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7311 P*

Effect of Rate of Stimulation and Work Performed on Lactic Acid and Hexosephosphate Changes.

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From the Department of Pharmacology, Washington University School of Medicine, St. Louis.

Sixty isometric contractions of isolated frog gastrocnemii against a tension lever at a rate of 10 per minute increased lactic acid 38 and hexosemonophosphate (as hexose) 28 mg. % over resting values of 23 and 51 mg. %, respectively. Sixty contractions at a rate of 60 per minute increased lactic acid 42 and hexosephosphate 56 mg. %. The average amount of work produced in the 2 series of experiments was practically the same. It may be seen that the average amount of lactic acid formed per 60 twitches was not appreciably changed by the rate of stimulation, while hexosephosphate increased much more with rapid than with slow stimulation. A 10 second isometric tetanus produced on an average an increase in lactic acid of 37 and in hexosephosphate of 64 mg. %. For an about equal amount of lactic acid produced, the hexosephosphate increase was greater in the tetanus than in the single twitches at a rate of 60 per minute.

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* P represents a preliminary, C a complete manuscript.

Owing to variations in the amount of work performed by individual muscles, it was possible to construct curves by plotting lactic acid or hexosephosphate against work. Lactic acid was proportional to the work both in single twitches and in tetani and the curves intersected zero work at the basal value of lactic acid as determined experimentally. Hexosephosphate was not proportional to the work; it rose rapidly with small amounts of work and less so as more work was produced, both in single twitches and in tetani.

Oxidative removal of lactic acid and hexosephosphate was excluded by performing some of the experiments in nitrogen. The difference in time between slow and fast stimulation (5 minutes) is too small to allow hexosephosphate to disappear, as shown in experiments in which the rapidly stimulated muscle was fixed 5 minutes after the end of stimulation. Hexosephosphate and lactic acid account for most of the glycogen broken down during contraction. The greater increase in hexosephosphate with rapid stimulation represents an extra breakdown of glycogen, while the relation of hexosephosphate to work suggests that as work is increased proportionately less glycogen is broken down.

7312 P

Antigenic Relationships of Diphtheria Bacilli and Derived Variants.

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The antigenic relationships between diphtheria bacilli and certain derived variants have been investigated by means of the complement fixation reaction.

Two strains of virulent diphtheria bacilli, *No. 19* and *Park No. 18* and avirulent variant strains derived from these strains were used in the experiments, and in addition, a strain of *C. hofmanni.*

Two of the variant strains, *Park No. 8 phage a* and *Park No. 8 phage b,* were derived from young cultures of *Park No. 8* to which specific bacteriophage had been added. Four of the variant strains were derived in various ways from the *No. 19* culture and are designated as follows: *19 'antiserum',* derived from culture in broth containing homologous antiserum, *19 'broth 45°C.'*, from broth culture incubated at *45°C.; 19 'LiCl'*, from culture in broth containing 0.5% lithium chloride; *19 'phenol'*, from culture in broth containing 0.1% phenol.

These variant organisms were found to be stable in type, grew very slowly in broth, and were completely lacking in fermentative ability. The variant colony was much smaller than the typical colony of diphtheria colony. Microscopically, the variant organisms were hardly distinguishable from the parent organisms. Usually they stained more solidly, but many barred and granular forms were observed.

Sera taken from some of the rabbits before immunization failed to fix complement except when large amounts of serum were used. The course of immunization consists of 8 to 10 injections of heat-killed organisms. The sera obtained were inactivated by heating them at 60-62°C. for 30 minutes.

The sera were used in 4 amounts: 0.025, 0.012, 0.006, and 0.003 cc. All antigens were used in such an amount that 4 times that amount was not anticomplementary. The smallest amount of serum giving a strong reaction with the homologous antigen was used as a base and the reactions in this amount with other antigens is reported in the tabulation.

TABLE I.

	Antiserum:								
	Park No. 8	Park No. 8, 'phage a'	Park No. 8, 'phage b'	19	19 'broth, 45° C.'	19 'antiserum'	19 'LiCl'	19 'phenol'	C. hofmanni
Antigen									
Park No. 8	+4	+3	—	+2	—	+3	+2	+2	—
Park No. 8 'phage a'	—	+4	—	+2	—	—	—	—	—
Park No. 8 'phage b'	+2	+3	+4	—	+1	+2	—	+2	—
19	—	—	—	+4	+1	+1	—	—	—
19 'broth 45° C.'	—	+3	—	+4	+4	—	—	—	—
19 'antiserum'	—	—	—	+4	—	+4	—	—	—
19 'LiCl'	—	—	—	—	—	—	+4	—	—
19 'phenol'	—	+2	—	—	+1	+2	—	+4	—
C. hofmanni	—	—	—	—	—	—	—	—	+4

Park No. 8 and *No. 19,* the virulent parent organisms are antigenically dissimilar. *Park No. 8* differs from both of its variants. *Park No. 8 phage a* antiserum reacts with all *Park No. 8* antigens, but the *phage a* antigen does not have the reciprocal relationship with *Park No. 8* antisera. *Park No. 8 phage b* antiserum reacts only with the specific antigen; however, the *phage b* antigen has a wide reactivity with both *Park No. 8* and heterologous antisera.

The *No. 19* immune serum has a wider range of reactions than *Park No. 8* antiserum. It has strong reactions with 2 of its variants, *19 'antiserum'* and *19 'broth 45°C'*, but does not react with the remaining 2. It also reacts to a slighter extent with the heterologous *Park No. 8* and *Park No. 8 phage a* antigens. It is interesting to note that its reaction with *19 'broth 45°C'*, was stronger than with the homologous *No. 19* antigen although the table does not indicate this reaction. The *No. 19* antigen reacted less intensely in the reciprocal relationships. *19 'LiCl'* and *19 'phenol'* bear no antigenic relationship to the *No. 19* strain. On the other hand their antisera react with *Park No. 8* antigen.

A multiplicity of antigenic factors may account for the diversity in antigenic variation amongst the variants. Dissociation seems to alter the antigenic structure of a single strain in a number of possible different ways. Variants from the same parent strain, though similar in morphological and cultural characteristics and derived in a similar manner, may differ in antigenic nature. The antigenic character of a variant may be so altered that it will react with heterologous strains or variants which will not react with the parent strain.

The literature affords conflicting reports regarding the antigenic relationship of the Hofmann bacillus to the diphtheria bacillus. Our results afford no indication of a relationship.

7313 P

Action of Veratrine on Medullated Nerve.

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From the Departments of Zoology and of Pharmacology, Washington University, St. Louis.

Fromherz[1] has recently investigated the effect of veratrine and other drugs upon the electrical response of medullated nerve, using a moving coil galvanometer as a measuring instrument. He detects little difference in response between normal and veratrinized nerve, if the nerve is suspended in oxygen throughout. If, however, veratrinized nerve be asphyxiated and allowed to recover in oxygen, he finds that the after-potential after a single shock or short tetanus may last as long as 10 minutes. We have reinvestigated the action of veratrine on the sciatic nerve of *R. pipiens* with the cathode ray oscillograph, and find that if the concentration is suitably adjusted to the temperature, typical veratrine after-potentials as measured out to one-half second can be obtained whether the nerve is in air or in oxygen. The results of Graham and Gasser[2] are therefore not due to partially asphyxial conditions, as suggested by Fromherz[1] and Hill.[5]

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[1] Fromherz, H., *J. Physiol.*, 1933, **79**, 67.

In order to study the after-potential throughout its course we have modified the usual oscillographic technique by employing a voltage amplifier of the direct-coupled type, especially designed to avoid amplifier drift (the theory of this amplifier has been published by Schmitt[3] and the details of the circuit as modified for electrophysiological use will be published elsewhere). By this method the resting potential is followed continuously so that due allowance for drift in resting potential can be made in estimating the duration of the after-potential. After a shock the potential changes can be followed for the first 3-4 seconds by photographing a slow sweep of the oscillograph beam; thereafter the potential may be measured every few seconds for as long as desired by reading the balancing potentiometer, using the oscillograph at high amplification as a null instrument. Preliminary experiments have revealed after-potentials lasting as long as 6 minutes after a single stimulation of veratrinized nerve in oxygen after previous asphyxiation. Without asphyxiation the potential seldom lasts longer than 10-30 seconds.

The action of veratrine on the gaseous metabolism of frog sciatic nerves at rest and in activity has also been studied. It has been found that the acceleration of the oxygen consumption reported by Schmitt and Gasser[4] usually reaches its maximal value within 10-30 minutes after exposure to veratrine, as determined by tipping the veratrine from a side arm in the Warburg respirometer onto the nerves lying in the main portion of the vessel. If after this veratrinization the nerves be asphyxiated and the oxygen consumption again followed after recovery, the new rate of respiration does not exceed that before asphyxiation. Hence it appears that veratrine permeates the nerve fairly rapidly under aerobic conditions and produces clear cut increases in oxygen consumption. It is difficult to interpret these experiments on the basis of the hypothesis of Fromherz[1] and of Hill[5] that veratrine is unable to penetrate the myelin sheath until after the sheath has been rendered permeable by a temporary lack of oxygen.

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[2] Graham, H. T., and Gasser, H. S., *J. Pharm. Exp. Ther.*, 1931, **43**, 163.

[3] Schmitt, O. H. A., *Rev. Sci. Instr.*, 1933, **4**, 661.

[4] Schmitt, F. O., and Gasser, H. S., *Am. J. Physiol.*, 1933, **104**, 320.

According to Hill the heat production following stimulation of veratrinized nerve in oxygen is not materially greater than normal unless the nerve has been previously asphyxiated. In this condition the heat production following a single shock may be a thousand times normal. Stimulation at a rate of 30 shocks per minute gave a heat production more than 30 times normal in one experiment. Under the conditions of our experiments this would correspond to an excess oxygen consumption of at least 15%. Schmitt and Gasser[4] have already observed definite increases in oxygen consumption of veratrinized nerves when stimulated at a rate of 2 per second. Preliminary experiments with an improved respirometer have demonstrated increases of 1-3% above the resting level with rates of stimulation of 16-30 shocks per minute, whether or not the nerve has been subjected to previous asphyxiation. The possibility therefore exists that a portion of the large heat production reported by Hill may have no counterpart in processes involving oxygen consumption.

7314 P

Normal and Experimentally Modified Mitotic Activity of Adrenal Gland in Guinea Pig.*†

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The mitotic activity of the thyroid gland in the guinea pig can be used as a quantitative measure of the stimulating effect exerted by various agencies on this organ.[1] In the following investigations we extended this method to the adrenal gland of the guinea pig and we determined the number of mitoses in this gland in the normal animal and under various experimental conditions. We soon found that there is a remarkable similarity in the mitotic activity in various sections through this organ at the same time, so that it is possible to obtain the measure of the mitotic activity of a gland by counting the mitoses found in 3 longitudinal sections through the center of this organ, each section being approximately 6 mikra thick. Counting the number of mitoses in a larger number of sections does not cause a significant alteration in the figures. The large majority of all mitoses are found either in the *zona glomerulosa* or in the outer portion of the *zona fasicularis* and it seems that, in the former area, mitoses occur especially in younger individuals. In these investigations, we excluded all animals which showed any sign of infection. In normal guinea pigs weighing 190-200 gm. the average number of mitoses per section is about 5; this number declines with increasing weight and age. In guinea pigs weighing 400-600 gm. it is about 1.5 mitoses per section. This series of normal animals includes 87 cases. There is no appreciable difference between males and females. The total number of mitoses in one adrenal gland of a guinea pig weighing 195 gm. was 1340, in the adrenal gland of a guinea pig weighing 340 gm., 930, and in a guinea pig weighing 515 gm., 165. Canalis stated that in sections of suprarenals from normal guinea pigs there might be 5 mitoses, in others as many as 12 per section.[2] Hoerr states that in sections cut 3 to 4μ one may expect to find on the average 2 to 8 mitoses in a longitudinal section through the center of a gland of a young adult guinea pig (350-450 gm.).[3]

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[5] Hill, A. V., *Proc. Roy. Soc.*, 1933, **113**, 386.

* These investigations were carried out with the aid of a grant for research in science made to Washington University by the Rockefeller Foundation.

† I wish to express my appreciation to Miss Helen Aff for her assistance in this experiment.

[1] Gray, S. H., and Loeb, Leo, *Am. J. Path.*, 1928, **3**, 257.

Keeping guinea pigs in a room with a temperature varying between 7° and 9°C. for from 8-25 days increased the number of mitoses to 15. The average of 3 controls was 3.6. An almost equal increase in mitotic activity was induced by underfeeding guinea pigs for periods of from 4-7 days, with a daily loss in weight varying between 2.5 and 9 gm. The average number of mitoses per section was 13. Average in 6 controls was 4.5.

Daily intraperitoneal injections of acid extract of anterior pituitary of cattle cause an increase in mitotic activity, which is most marked if, in the course of 6 days, increasing amounts of extract are injected on successive days. The average number of mitoses of 6 guinea pigs thus treated was 50.5. If daily injections of 1 cc. acid extract are given, the increase is much less marked and the number returns almost to normal, if the injections are continued over a long period of time; this corresponds to an analogous effect in the case of the thyroid gland.[4] Also inoculations of anterior pituitary glands from guinea pig and rabbit caused a marked increase in the number of mitoses. Daily inoculations of one or 2 anterior pituitary glands from guinea pigs led to a rise in the average number of mitoses to 38. A similar increase was induced by daily inoculations of rabbit anterior pituitary. This figure was still higher (an average of 69 mitoses) in a series in which the administration of rabbit anterior pituitary was preceded by a short period of underfeeding. On the other hand, if in a first period, acid extract was injected daily and, in a subsequent period, guinea pig or rabbit anterior pituitary glands were inoculated, the rise in the mitotic activity was much less. This is perhaps due to an antagonism that exists between the hormones predominant in the anterior pituitary glands of rabbits, rats and guinea pigs on the one hand and in extract of cattle anterior pituitary on the other hand. To our surprise we noted that after inoculation of rat anterior pituitary into guinea pigs, only an exceptional rise in mitotic activity was induced in the adrenal gland of the guinea pig. This apparent relative ineffectiveness of rat anterior pituitary needs further analysis.

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[2] Canalis, P., *Internationale Monatsschr. f. Anat. und Physiol.*, 1887, **4**, 312.

[3] Hoerr, W., *Am. J. Anat.*, 1931, **48**, 139.

We may then conclude that various hormones of the anterior pituitary cause a rise in the mitotic activity of the adrenal gland of the guinea pig, and that this effect seems to be independent of the action of these hormones on the thyroid gland, as evidenced by the fact that the guinea pig anterior pituitary, which has no or only a very slight effect on the thyroid gland,[5] may have a very marked effect on the adrenal gland.

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[4] Loeb, Leo, and Friedman, Hilda, PROC. SOC. EXP. BIOL. AND MED., 1931, **29**, 172.

[5] Loeb, Leo, *Endocrinology*, 1932, **16**, 129.

7315 C

Effects of Anterior Pituitary Extracts of Cattle on Carbohydrate Metabolism in the Guinea Pig.*

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From the Department of Pathology, Washington University School of Medicine, St. Louis.

In view of preceding observations that injections of anterior pituitary glands of cattle cause a marked stimulation of the thyroid gland in guinea pigs,[1] leading to a loss of weight by the animals[2] and to an increase in the basal metabolic rate,[3] it was of interest to investigate the effects of such extracts on the carbohydrate metabolism.

Artundo and Mazzocco[4] found no change in the glycogen reserves of white rats after injections of fresh rat hypophysis in saline. Eitel and Loeser[5] have reported studies on guinea pigs, using anterior pituitary extracts containing thyroid stimulating hormone. They noted a fall in liver glycogen, reaching minimal values in 11 days, with a slow increase subsequently. They report no change in muscle glycogen. Our results are somewhat different in certain respects. We likewise used guinea pigs, which had served for the large majority of experiments carried out by Loeb and Siebert.[6] Male guinea pigs (190-200 gm.) kept under optimal conditions were used in all experiments. The extract was that used previously in this department.[7] Injections were made intraperitoneally with sterile precautions. The animals were killed 24 hours following the last injection and glycogen determined by a modification of the Pflüger method,[8] blood sugars after Shaffer and Somogyi.[9] Two or more animals were employed for each interval recorded and all glycogen determinations were made in duplicate. The points in the accompanying graphs represent averages.

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*These investigations were carried out with the aid of a grant for research in science made to Washington University by the Rockefeller Foundation.

[1] Loeb, Leo, and Bassett, R. B., PROC. SOC. EXP. BIOL. AND MED., 1929, **26**, 860.

[2] Loeb, Leo, and Friedman, Hilda, *Ibid.*, 1931, **29**, 14.

[3] Siebert, Walter J., and Smith, R. S., *Ibid.*, 1930, **27**, 622.

[4] Artundo, A., and Mazzocco, P., *Compt. Rend. Soc. Biol.*, 1930, **105**, 713.

[5] Eitel, H., and Loeser, A., *Arch. Exp. Path. and Pharm.*, 1932, **167**, 381.

[6] Loeb, Leo, and Siebert, Walter J., *Loc. cit.*

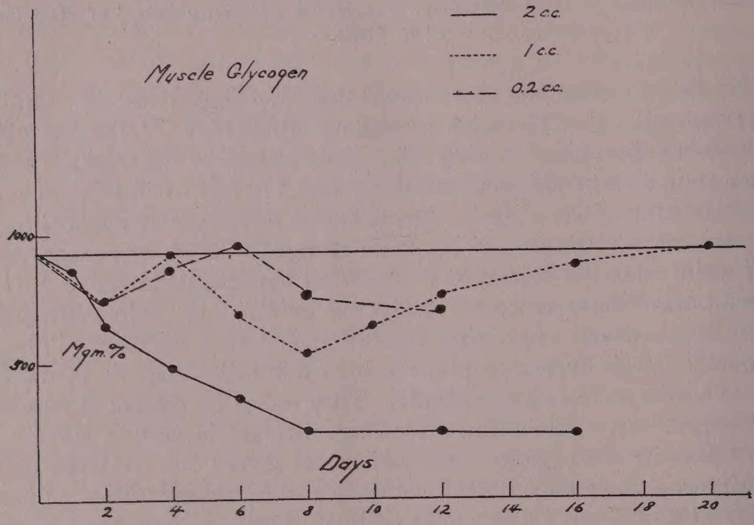
[7] Loeb, Leo, and Bassett, R. B., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 490.

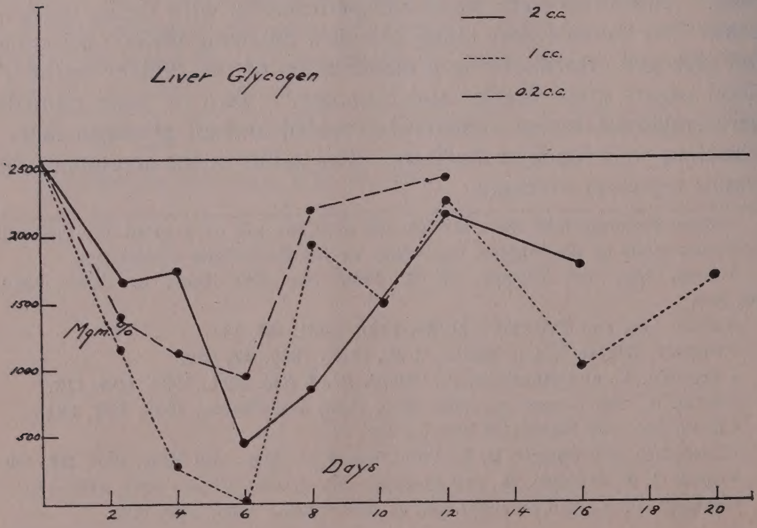
[8] Good, C. A., Kramer, H., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 485.

[9] Shaffer, P. A., and Somogyi, M., *J. Biol. Chem.*, 1933, **100**, 695.

The following normal values were established:

		(Eitel and Loeser)
Liver glycogen	2540 mg. %	2590 mg. %
Muscle glycogen	930 mg. %	520 mg. %
Blood sugar	90-126 mg. %	68-120 mg. % (Hagedorn-Jensen)





Liver glycogen determinations were made after injections of 2 cc., 1 cc., and 0.2 cc. of acid extract. In each case, a rapid decline in the liver glycogen occurred, reaching a minimum in about 6 days. A slow recovery then occurred, notwithstanding continued injections. In no case did the liver glycogen return completely to normal. With injections of 2 cc. and 1 cc., the effects were similar, while with 0.2 cc. a less marked response obtained. The character of the liver glycogen curve was essentially the same in each case. No significant change in liver weight could be detected. The animals injected with the larger amounts tended to lose weight, as recorded previously.[1]

With injections of 2 cc. daily, a fall in muscle glycogen (samples taken from thigh muscles) was noted. A subnormal level persisted during the course of the experiment. With 1 cc. and 0.2 cc. injections no significant change in muscle glycogen occurred.

Blood sugar determinations made concurrently with the glycogen estimations were not consistent, ranging from 92-384 mg. %. However, in one-third to one-half of the determinations, values were obtained exceeding the highest normal levels. This suggested that possibly the time factor might enter as a variable in these determinations and that the optimum might have been passed in many cases when the blood samples were taken 24 hours following the last injection. We, therefore, determined the blood sugar values as early as 4, 8 and 13 hours following a single injection of acid extract. The following figures were obtained: After 4 hours, 170 mg. %; after 8 hours, 232 mg. %; after 13 hours, 204 mg. %; after 24 hours, 138 mg. %.

Our results are in agreement with those of Eitel and Loeser in that we found a rather prompt fall in liver glycogen following the initial injections. However, our results differ in the following aspects:

(1) In our experiments, the liver glycogen reached a minimum in each case at 6 days, whereas according to Eitel and Loeser the minimum is reached in 11 days.

(2) We found a rather rapid recovery of the liver glycogen to slightly subnormal levels, notwithstanding continuation of the injections; whereas they report continued low glycogen values during the period of injections. Our results may perhaps be best explained by assuming a high activity of the extract employed and a rapid compensation on the part of the animal. Our curves are in agreement with the findings of Loeb and Bassett[1] that the maximum stimulation of the thyroid gland, as evidenced by the curve of the

mitotic activity, is reached about 6 days following the initial injection, or is reached during the first week, to be followed by a decrease.

(3) We find considerable quantitative differences in the diminution of liver glycogen depending upon the amount of extract given.

(4) We note a definite effect on muscle glycogen, provided sufficiently large doses have been injected.

As to the blood sugar level, a rise has been observed in the experiments of Houssay, *et al.*,[10] in the dog; and by Barnes; Evans; and Baumann and Marine[10] in the rabbit. However, these authors have obtained their effects after repeated injections of large amounts of anterior pituitary extracts. This is in contrast with the sharp rise and fall of blood sugar values observed in our preliminary experiment. This matter is being investigated further.

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[10] Houssay, B. A., Biassotti, A., Rietti, C. T., *Compt. Rend. Soc. Biol.*, 1932, **111**, 479. Barnes, B. O., Reported by E. I. Evans, PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1370. Evans, E. I., *Loc. cit.* Baumann, E. J., and Marine, D., *Ibid.*, 1932, **29**, 1220.

New York Meeting.

New York Academy of Medicine, April 18, 1934.

7316 P

Protection Afforded by Certain Vegetable Oils against Nutritional Encephalomalacia of Chicks.[*]

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For several years, attempts have been made to protect chicks against nutritional encephalomalacia[1] (1) by supplementing Diet 108[†] with various natural foodstuffs; (2) by varying the proportion of the ingredients. A summary of these experiments is given in Table I.

Although there have been indications that the addition of certain foodstuffs such as wheat, corn, lettuce, spinach and grass conferred partial protection, the results have been by no means consistent. Various modifications in the proportion of the mineral and organic ingredients of Diet 108 have likewise failed to induce complete protection. It has been noted that reduction of the lard content regularly lowers the incidence of the disease, but even complete omission of lard from the diet does not give absolute protection. This indicates that the disease is not due primarily to toxicity of the lard.

When certain vegetable oils were substituted for lard in Diet 108, all the chicks remained free from the disease. Experiments demon-

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[*] This work was aided in part by the Research Grant from the Chemical Foundation to the Department of Biological Chemistry.

[1] Pappenheimer, A. M., and Goettsch, M., *J. Exp. Med.*, 1931, **53**, 11; 1933, **57**, 365.

[†] Diet 108 has the following composition:

	%		%
Casein	20.5	Cod liver oil	2.0
Cornstarch	20.0	Yeast	5.0
Lard	21.0	McCollum salts	6.5
Skimmed milk powder	15.0	Paper pulp	10.0

TABLE I.

Diet	No. of chicks	No. with microscopic lesions	% microscopic lesions
Natural foods—cereals, milk products, minerals, yeast, cod liver oil	166	2	1.2
Same diets, autoclaved 6 hours 120°, with and without added yeast	38	0	0.0
Diet 108	271	164	60.0
Diet 108 with greens			
Lettuce, 2-6 gm. daily per chick	58	10	17.0
Spinach, 5 " " " "	28	3	11.0
Grass, 2-5 " " " "	40	4	10.0
Cabbage, 5 " " " "	14	8	57.0
Dried alfalfa meal, 10%‡	29	17	58.0
Diet 108 with cereals and cereal products‡			
Whole wheat, 20%	104	20	19.0
Wheat sprouts, 5 gm. daily per chick	31	5	16.0
Wheat products, equivalent to 40% wheat	100	46	46.0
Yellow corn, 20%	16	3	19.0
Polished rice, 25%	13	10	77.0
Diet 108 with animal tissues and products‡			
Meat scraps, 20%	15	11	73.0
Beef liver, 5 gm. daily per chick	15	9	60.0
Calf brain, 0.5-5 gm. daily per chick	75	42	56.0
Egg yolk, 1 cc. daily per chick	15	4	27.0
Lecithin	17	9	53.0
Changes in the amounts of the ingredients present in Diet 108‡			
Yeast, 20%	8	8	100.0
Cod liver oil omitted, chicks irradiated; in one diet butter added	42	25	60.0
Minerals increased to equal value of natural foods diet	21	14	66.0
$CaCO_3$ added to improve Ca/P ratio	34	12	35.0
Skimmed milk omitted	41	10	24.0
Change in lard-cornstarch ratio:			
Per cent lard — Per cent cornstarch			
0.0 — 53.4	28	8	29.0
3.0 — 45.0	17	4	25.0
21.0 — 20.0 (Diet 108)	271	164	60.0
33.7 — 0.0	15	8	57.0

‡ Diets prepared so as to maintain constant nutritive ratio.

TABLE II.

Diet	No. of chicks	No. with microscopic lesions	% microscopic lesions
Substitution of vegetable oils for lard in Diet 108			
Wesson oil, cotton-seed	13	0	0.0
Crisco, hydrogenated cotton-seed oil	28	0	0.0
Mazola, corn-oil	30	0	0.0
Peanut oil	14	0	0.0
Olive oil	14	3	21.0

strating this are presented in Table II. Thus far Crisco, Wesson Oil, Mazola and peanut oil at the level of 20% have afforded complete protection. Olive oil has been less efficacious. Fractionation of these oils is now being carried out in the attempt to gain further knowledge as to the nature of the protective factor.

7317 C

Experiments on Vitamin G Concentration and Possible Supplementary Relationships with the Vitamin G Deficient Diet.

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From the Department of Chemistry, Columbia University.

Attempts to concentrate the vitamin G of food materials[1, 2, 3] before completion of the work here reported, have been complicated by the postulation of a multiplicity of factors in vitamin G.[4–14] While the question of this possible multiple nature of the vitamin still remains very unsettled, much progress has been made in the concentration of the vitamin.[15, 16, 17] The present paper reports additional evidence of the existence of some unknown variant in vitamin G research and describes a method of concentration of vitamin G which is being used as an initial procedure in the subsequent concentration work of Booher.[17]

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[1] Naryanan, B. T., and Drummond, J. C., *Biochem. J.*, 1930, **24**, 19.

[2] Guha, B. C., *Biochem. J.*, 1931, **25**, 945.

[3] Supplee, G. C., Kahlenberg, V. J., and Flanigan, G. E., *J. Biol. Chem.*, 1932, **65**, 317.

[4] Palmer, L. S., and Kennedy, C., *J. Biol. Chem.*, 1927, **74**, 591.

[5] Palmer, L. S., and Kennedy, C., *J. Biol. Chem.*, 1927, **75**, 619.

[6] Palmer, L. S., and Kennedy, C., *J. Biol. Chem.*, 1928, **76**, 591.

[7] Hunt, H. C., *J. Biol. Chem.*, 1928, **78**, 83.

[8] Hunt, H. C., *J. Biol. Chem.*, 1928, **79**, 723.

[9] Coward, K. H., Key, K. M., and Morgan, B. G. E., *Biochem. J.*, 1929, **23**, 695.

[10] Coward, K. H., Key, K. M., Morgan, B. G. E., and Cambden, M., *Biochem. J.*, 1929, **23**, 913.

[11] Chick, H., Copping, A. M., and Roscoe, M., *Biochem. J.*, 1930, **24**, 1748.

[12] Chick, H., and Copping, A. M., *Biochem. J.*, 1930, **24**, 1764.

[13] Williams, G. Z., and Lewis, R. C., *J. Biol. Chem.*, 1930, **89**, 275.

[14] Stiebeling, H. K., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1155.

[15] Kuhn, R., György, P., and Wagner-Jauregg, T., *Ber. chem. Ges.*, 1933, **66**, 317.

[16] Kuhn, R., György, P., and Wagner-Jauregg, T., *Ber. chem. Ges.*, 1933, **66**, 576.

[17] Booher, L. E., *J. Biol. Chem.*, 1930, **102**, 39.

The method used in the evaluation of various materials for their vitamin G content was essentially that of Bourquin and Sherman.[18] During this investigation the small anti-coprophagy harness previously described[19] was first used to minimize the error due to coprophagy. In a group of 37 animals, half of which were harnessed, the average total gain at the end of the fifth week for the group restricted in respect to coprophagy was 18.8 ± 1.2 gm., while in the parallel group, unrestricted in respect to coprophagy, the corresponding gain was 29.4 ± 2.2 gm. (Deviation measure = P.E.). Observed activity of the animals and the greater precision in growth records of the harnessed animals indicates that the harness *per se* did not restrict growth.

Concentration Experiments. A concentrate of vitamin G from dried skimmed milk powder was obtained as follows: 500 gm. of air dried skimmed milk powder were refluxed successively and with constant agitation by means of a stream of purified nitrogen with 2000 cc., 1250 cc., and 750 cc. of neutral boiling 93-94% ethyl alcohol (by weight) for 30, 30, and 15 minutes respectively. The 3 alcoholic extracts combined, dried and analyzed showed a nitrogen and phosphorus content of 4.44 ± 0.02% and 0.47 ± 0.01% respectively (precision measure ± A.D.). The potencies of the alcoholic extract and of the extracted milk powder were measured and the results summarized in Table I (a, b, c). The alcoholic extract (5% of the solids of the original milk powder) was found to be 10 times as rich in vitamin G as the original milk powder. Half of the total activity was in the alcoholic extract and half in the extracted milk powder.

Evidence of Some Variant in Vitamin G Testing. A further attempt was made to concentrate the vitamin by reducing the alcoholic extract to a small volume by distillation under reduced pressure and adding to this concentrated extract an equal volume of ethyl ether. A precipitate was obtained (analysis: nitrogen = 3.49 ± 0.05%, phosphorus = 0.255 ± 0.005%; precision measure = A.D.) which was only about 4 times as rich in vitamin G as the original milk powder, Table I(d); whereas the alcoholic extract had shown a ten-fold concentration. The loss of activity could not be accounted for in the alcoholic filtrate remaining after ether precipitation. Attempts to ascertain any supplementary relation (evidence of vitamin G "factors") between the ether precipitate and the alcohol

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[18] Bourquin, A., and Sherman, H. C., *J. Am. Chem. Soc.*, 1931, **53**, 350.

[19] Page, J. W., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 87.

TABLE I.

Supplements	Amt. in gm. per day	No. animals	Average gain in gm. per week over a 5 weeks' experimental period: Short depletion (21-28 days)	Long depletion (56 days)
a. Untreated milk powder	0.3	4	4.1 ± 0.5	
b. Alcoholic extract	0.03	5	4.3 ± 0.3	
c. Alcohol extracted milk powder	0.3	7	2.0 ± 0.5	
d. Ether precipitate	0.07	7	4.2 ± 0.2	
e. Alcohol extracted milk powder	0.3	4		—0.1 ± 0.4 (1 dead)
f. Ether precipitate	0.07	4		6.4 ± 0.3
g. Ether precipitate plus Alcohol extracted milk powder	0.035 0.15	4		5.7 ± 0.4
h. Negative controls		17	—1.1 ± 0.3	
		3		1 animal survived experimental period

extracted milk powder by the method[14, 20] of feeding each material alone, and in parallel a combination of ½ the amounts each of ether precipitate and of alcohol extracted milk powder as was fed alone, were not productive of results which could explain the loss of potency in the ether precipitate.

It was, however, noticed that animals continued beyond the usual 5-week experimental period often showed a sharp decline in body weight and this especially when the source of vitamin G was the alcohol extracted milk powder; suggesting that at this time some further growth essential had become necessary through depletion of the bodily stores of the animal of that essential. A series of supplementation experiments were conducted using animals that had been carried through a long depletion period of 56 days (instead of the usual 21-28 days) on the vitamin G deficient diet alone. Table I (e, f, g) gives the results of this work. The same amount of alcohol extracted milk powder as a vitamin G supplement which produced fair growth after 4 weeks' depletion was practically inactive in producing growth after 8 weeks' depletion, Table I(e). However, this extracted milk powder fed in combination with the ether precipitate enhanced the growth-producing power of the latter; thus the combination produced the same growth as that resulting from feeding twice as much of the ether precipitate alone as a source of vitamin G, Table I(f, g), Figure 1.

In the present state of our knowledge, the only satisfactory explanation of this supplementary relation between the 2 fractions would seem to be that a long depletion period (56 days) results in depleting the bodily reserves of the rats of a second limiting factor.

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[20] Bisbey, B., Dissertation, Columbia University, 1930.

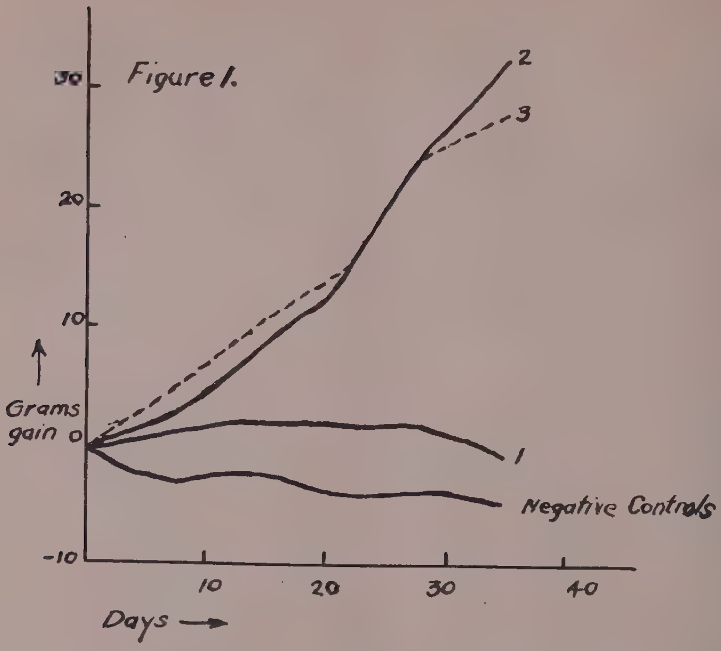

FIG. 1.

Comparison of growth of rats receiving as supplement to a vitamin G deficient diet:

1—0.3 gm. daily of alcohol extracted milk powder.
2—0.07 gm. daily of "ether precipitate."
3—0.15 gm. daily of alcohol extracted milk powder plus 0.035 gm. daily of "ether precipitate."

after 56 days' depletion on a vitamin G deficient diet.

The first limiting factor is carried by both the ether precipitate and the alcohol extracted milk powder, the second factor by the ether precipitate only. This interpretation is applied only as a suggestive hypothesis which best fits the experimental facts so far available.

Summary. 1. A ten-fold concentration of the vitamin G of dried skimmed milk powder has been effected by extraction of the milk powder with hot 93-94% (by weight) ethyl alcohol. Half of the vitamin G activity remained intact in the extracted residue and half was accounted for in the alcohol extract. 2. Evidence for some variant in vitamin G testing has been obtained. This variant may be interpreted as a second limiting substance in vitamin G testing which is preferentially precipitated by addition of ethyl ether to the alcoholic extract of skimmed milk powder. 3. The practicability of the anti-coprophagy harness for increasing the precision of vitamin

G testing is verified by its successful use throughout this investigation.

7318 C

Tuberculosis Induced in the Tadpole by Feeding.*

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Aronson[1] isolated a strain of tubercle bacillus from an iguana which was found dead in the Philadelphia Zoo. A number of tubercles were seen in the lung of this animal and also in the liver. The spleen contained no gross tubercles. A single tubercle was found in the upper pole of the left kidney. In guinea pigs injected intracutaneously with this culture Aronson found that a small ulcer occurred at the site of inoculation. The glands became enlarged and succulent but went on to healing. The organism was found to be pathogenic for the chameleon and salamander and also for the frog. It was not pathogenic for the snake. As regards the frog Aronson's findings have been confirmed by Holly[2] and by the writers with a transfer of this organism which Aronson sent to us sometime ago for further study.

Examination of stained slide preparations reveals the organism to be acid- and alcohol-fast. Ziehl-Neelsen stained vertical sections from growing colonies made with a technique reported by us[3] show some non-acid-fast rods in addition, but the tiny non-acid-fast rods and granules so conspicuous in vertical sections of human and bovine tubercle bacillus colonies are entirely lacking. It would seem then that this organism multiplies principally by simple fission. According to Henderson and Aronson[4] the organism is culturally and serologically identical with *Mycobacterium marinum* which was isolated from salt water fish.

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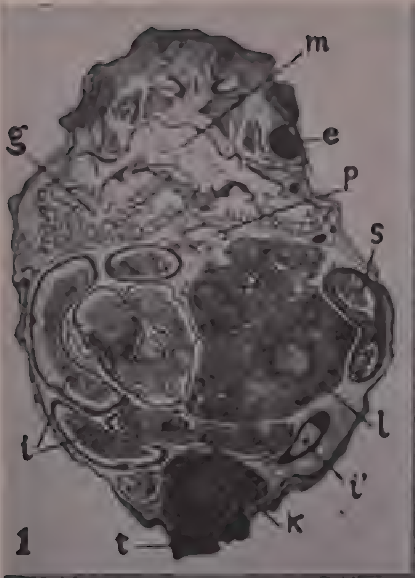
* This study was made in cooperation with the Research Board, National Tuberculosis Association.

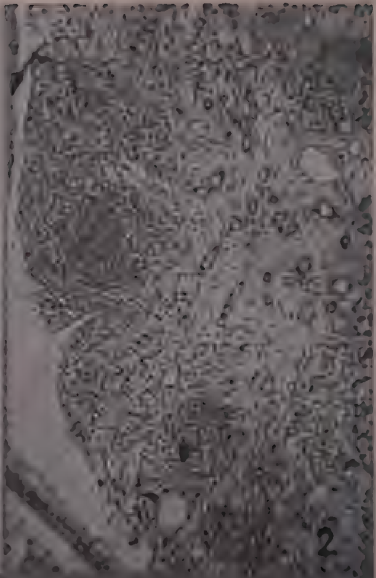
[1] Personal Communication, Dr. Joseph Aronson, Phipps Institute, Philadelphia, Pa.

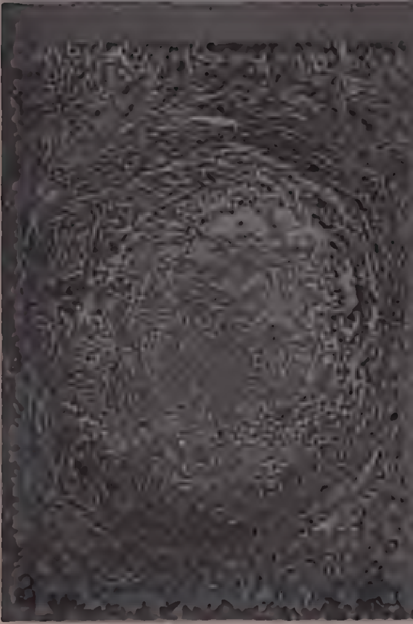
[2] Personal Communication, Miss Pearl Holly, New York City Department of Health.

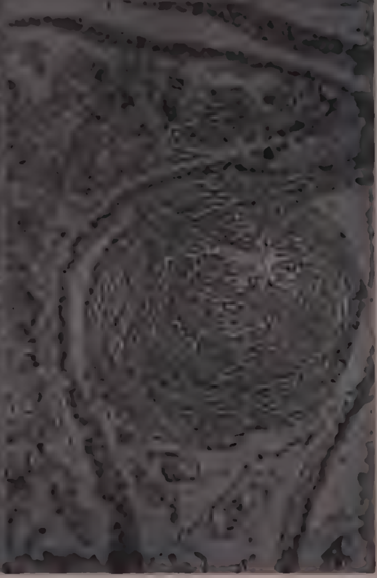
[3] Kahn and Nonidez, Proc. Soc. Exp. Biol. and Med., 1933, **30**, 577.

[4] Personal Communication.









All figures are unretouched photomicrographs from sections stained with hematoxylin and eosin.

FIG. I. Frontal section of infected tadpole (tail not included) showing large

In view of the pathogenicity of this organism for frogs it occurred to the writers to determine whether a similar situation existed for tadpoles. If this proved to be the case the tadpole would make a valuable experimental animal for certain problems as the entire animal may be sectioned and all of the organs examined in the same creature at the same time. It would also permit investigations concerning the reaction of embryonic tissue toward infection by a member of the *Mycobacterium* group.

Tadpoles of the leopard frog *(Rana pipiens)* were kept in sterile aquarium water contained in Stender dishes having a diameter of 10 cm. and a depth of 4.5 cm. A large loopful of a 5-day-old culture was scraped from a slant of Petroff's egg medium and deposited in the water. Possibly in view of the bright orange color no difficulty was experienced in inducing the tadpoles to feed upon this material. After feeding on the bacteria the tadpoles were removed to other Stender dishes also containing sterile aquarium water.

The infected tadpoles were fixed in alcohol or in a mixture of alcohol-chloral hydrate-formaldehyde, which latter mixture gave better fixation. The tails were cut off after fixation and the whole body dehydrated, embedded in paraffin and cut into frontal serial sections. (7-10μ thick). The entire animal with the exception of the tail was thus included on the slides. (Fig. 1.)

The sections were stained with hematoxylin-eosin (for histological details) or with the Ziehl-Neelsen technique.

Tadpoles given the bacteria as their only food for several days were soon overwhelmed without showing lesions of tuberculosis although numerous acid-fast organisms were found in liver and lung. Those fed twice with an interval of several days between feedings developed typical tuberculosis in the liver and intestine.

In one of the experiments 5 tadpoles were fed tubercle bacilli for one day; they were given yolk from a hard-boiled egg the 4 following days. On the fifth day they were fed tubercle bacilli again. One tadpole died during the night and could not be preserved for histological study. The other 4 were killed as follows: No. 1, killed 10 days after first, 5 days after second feeding. No. 2, killed 12 days after first, 7 days after second feeding. Nos. 3 and 4, killed 29 days after first, 24 days after second feeding.

tubercles in liver (l). e, eye; g, gills; i, small, and i′, large intestine; k, kidneys (mesonephroi); m, mouth cavity; p, transition from pharynx into oesophagus; s, stomach; t, root of the tail.

FIG. 2. Early tubercles at the portal of the liver. The small ducts appearing in cross section are hepatic ducts.

FIG. 3. Advanced tubercle with necrotic center, liver.

FIG. 4. Tubercle developed in submucosa of the small intestine.

While only 2 feedings of bacteria were given it is possible that the tadpoles ingested these organisms from the feces, for the organisms seemingly reproduce in the intestinal tract as they remain there for a long time after feeding them has been discontinued.

Study of the sections of the 4 tadpoles revealed the following conditions: No. 1, tubercle bacilli present in liver; first stages of formation of the tubercles already seen. Also larger tubercles in submucosa of intestine. No. 2, numerous tubercles present in liver, a few with beginning necrosis. No. 3, early, and advanced tubercles with necrotic centers in liver. No. 4, mostly early tubercles, a few with beginning necrosis in liver. A few tubercles also seen in the spleen, containing bacilli.

The structure of the tubercles does not differ in any essential point from those found in mammals. In the largest ones the center appears necrotic and contains acid-fast bacteria. (Fig. 3.) Around the necrotic center there are numerous epithelioid cells, as in the mammal, but no giant cells. The smaller tubercles, whether in the liver (Fig. 2) or in the intestine (Fig. 4) also contain acid-fast bacteria enclosed within phagocytic cells not yet degenerated. The area of the intestinal wall close to the tubercles shows marked changes in its cells and an abundance of tissue phagocytes.

Aside from the intestinal wall, the liver and the lungs, no other organ shows either lesions or bacteria. The stomach wall appears intact and organisms do not occur within the glands, which have wide ducts opening on the surface of the mucosa. Bacteria were also absent in the spleen (except in tadpole No. 4), pancreas, kidneys (mesonephroi), thymus and gonads.

Of special interest are the large numbers of acid-fast organisms found in the alveoli and central cavity of the lungs. The latter in the amphibia are much simpler than the corresponding organs of birds and mammals. Each is a sac with a wide lumen and diverticula representing the alveoli. In the tadpole the lungs extend from the glottis (representing the larynx) to the posterior end of the body and are in contact with the organs of the abdominal cavity since there is no diaphragm.

That the bacilli present within the lungs were not inhaled by the tadpoles is shown by the following facts: 1. Numerous bacilli were found in the tadpoles in which the lungs had not reached full development, the respiratory exchanges taking place through the gills. In these young tadpoles the glottis is closed and no air enters the lungs. 2. Our observations indicate that the tubercle bacilli present in the lung are brought there by migrating phagocytes. Accordingly, the bacilli occur among degenerated cells most of which are phagocytes, though there are also a few desquamated epithelial cells. Phagocytes containing tubercle bacilli were observed crossing the walls of the lung prior to their entrance in the lung cavity. 3. In older tadpoles, breathing through their lungs, the cellular contents of the lung cavity are much reduced; free cells and bacteria are presumably eliminated through the movement of the cilia of the epithelial cells lining part of the walls of the central cavity of the lung. The alveolar walls are much thinner in this case, and still contain large numbers of acid-fast bacteria.

Another feature of great interest from the standpoint of the path followed by the tubercle bacilli during the infection is their total absence from the blood. We have carefully examined the contents of the heart and of the larger veins, especially the inferior vena cava and the branches of the portal vein in the liver. In no case have we been able to identify free tubercle bacilli in the plasma or enclosed within leucocytes. Furthermore, in some areas in which extravascular leucocytes were present (submucosa of the intestine in the vicinity of tubercles) they did not contain any bacteria.

From our observations it follows that phagocytosis of the bacilli is accomplished only by tissue phagocytes similar to, or identical with, the histiocytes of the mammals.

A point now being investigated by the writers is the exact mode of passage of the bacilli from the intestinal cavity to other organs of the tadpole. Once the intestinal barrier has been surmounted the indications are that they follow the lymphatics or enter the peritoneal cavity, and in the latter case they may enter the organs by crossing through their outer serous coat.

In concluding we wish to emphasize the main point of the present communication, that is, that typical tuberculous lesions may be produced by feeding, and that at least in the case of tadpoles still breathing through their gills large numbers of tubercle bacilli may reach the lungs by an indirect route, being dumped there, so to speak, possibly to facilitate their elimination from the body. The presence of bacilli in the lungs of the tadpoles cannot be attributed, therefore, to direct inhalation of bacilli.

7319 P

Pacinian Corpuscles in the Mesentery and Their Relation to the Vascular System.*

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From the Eldridge Reeves Johnson Foundation for Medical Physics, University of Pennsylvania.

The question has frequently been raised[1, 2] as to whether there are vascular zones other than the carotid sinus and the aorta that make an afferent contribution to cardiovascular reflexes. A region that might well be suspect is the mesenteric area and we have accordingly investigated the problem of the afferent nerve discharge therefrom especially with relation to blood pressure.

As a means of approach impulses were recorded in the peripheral end of the splanchnic nerve or in its terminal branches which run along the mesenteric arteries. Characteristically there is a discharge of impulses synchronous with each pulse as in Fig. 1, although in certain cases there is a considerable amount of activity in the intervals between succeeding systoles. The general picture of this afferent discharge is similar to that observed[3] in the carotid sinus and aortic nerves.

The modifications effected in the discharge by variations in intravascular pressure are likewise similar to those found in the case of the carotid sinus and aorta although less constant and predictable.

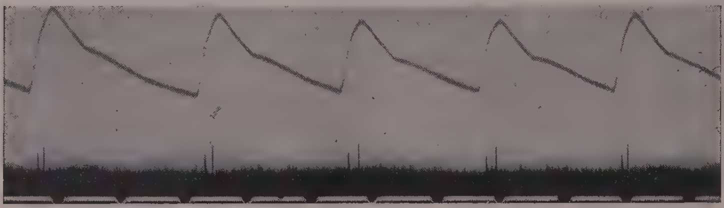

FIG. 1.

Afferent impulses in several fibers of splanchnic nerve. Upper record: carotid pulse; middle: action potentials; bottom: time in 1/5 secs.

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* The expenses of this investigation have been in part defrayed by a grant from the Committee on Scientific Research of the American Medical Association.

†Godey Fellow in Medicine.

[1] Heymans, C., Bouckaert, J. J., and Regniers, P., *Le Sinus Carotidien*, Paris, G. Doin, 1933.

[2] Katz, L. N., and Saphir, O., *Am. J. Physiol.*, 1933, **104**, 1.

[3] Bronk, D. W., and Stella, G., *J. Cell. and Comp. Physiol.*, 1932, **1**, 1.

When a portion of the mesenteric artery is perfused at steady pressures it is found that the end organs begin to discharge at a certain minimal pressure, the impulse frequency and number of active end organs increasing with further increases in pressure. In the intact preparation the degree of discharge is largely influenced by the magnitude of the pulse pressure, its rate of change, and especially by the blood volume. An increase in the latter increases the frequency of the afferent impulses. An increase in mean blood pressure, especially if the previous pressure is not too high, also produces an increase in the discharge although in some cases an increase in blood pressure resulting from an injection of adrenalin gave an initial decrease in the number of impulses. This may be due to a change in the relation of the vessels to the end organs.

In considering the question as to what type of end organ is responsible for this activity we were impressed by the proximity of many Pacinian corpuscles to the mesenteric arteries. It has been found[4] that such sense organs when associated with tendons are receptors for mechanical stimuli and it occurred to us that some of these in the mesentery might therefore be stimulated by movement of the vessel walls. This is the case, for in a number of experiments it has been possible to eliminate the pulsatile discharge in a small mesenteric nerve by removing one after another of the corpuscles in the field supplied by that nerve. It may be that some of the afferent impulses in the splanchnic nerve synchronous with the pulse originate in end organs other than Pacinian corpuscles, but certainly the latter are chiefly responsible.

Thus far we have no satisfactory evidence as to the reflex effects of these afferent impulses from the Pacinian corpuscles, but it is reasonable to assume that such an afferent discharge modified as it is by blood volume, mean blood pressure and pulse pressure may influence the activity of the cardiovascular centers.

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[4] Adrian, E. D., and Umrath, K., *J. Physiol.*, 1929, **68**, 2.

7320

Experiments on Sensitization of Guinea Pigs with Simple Chemical Compounds.

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The sensitization of guinea pigs with simple compounds such as primulin,[1] salvarsan,[2] and p-phenylene diamine[3] has been studied by several authors, but the development of the subject has been somewhat hindered by difficulties encountered in reproducing some of these experiments.[4]

As already observed by Mayer, we found that guinea pigs rubbed repeatedly with salve containing p-phenylene diamine showed increased redness and scaling, in comparison to controls. Furthermore, increased sensitivity was obtained by the administration of a salve containing p-nitroso-dimethylaniline and by repeated intracutaneous injections of a saline solution of p-phenylene diamine, and some such effects were noticed, also, after injecting p-nitroso-dimethylaniline and p-amino-phenol, using approximately 1 mg. of the substances per injection.

Following this, smaller doses of 0.02 mg. were injected on the back twice a week over a longer period, a method similar to that advocated by Kolle[5] for the production of hypersensitiveness to salvarsan, in animals. With this method definite sensitization effects were obtained, besides salvarsan, with other compounds such as p-phenylene-diamine and p-nitroso-dimethylaniline. Thus, on reinjection on the sides about 7 days after the last injection of a course consisting of up to 20 injections at one or 3 day intervals, a number of the guinea pigs showed pinkish, more or less elevated areas within 24 hours, averaging ¾-1 cm. in diameter. Likewise, with 1:2:4 dinitro-chlorbenzene* distinctly increased reactions, noticeable even after less than 10 injections, were observed in most of the guinea pigs after daily intracutaneous injections of the substance. In a preliminary experiment with suberanilic-resorcine dye,[6] 2 out of 6 animals treated in the manner described showed definite reactions, so that one may assume that sensitization is possible with this substance, also. White guinea pigs were used throughout. Intracutaneous injections of 1/50 mg. of the compounds in 0.1 cc. saline were used for the tests as well as for sensitization except in the case of dinitro-chlorbenzene, where doses of 1/400 mg. were injected. Normal animals used as controls showed no definite or much weaker reactions than those described.

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[1] Bloch, B., and Steiner-Wourlisch, A., *Arch. f. Dermat. u. Syph.*, 1926, **152**, 283; 1930, **162**, 349.

[2] Swift, H., *J. A. M. A.*, 1912, **59**, 1236; Frei, W., *Klin. Wochenschr.*, 1928, **7**, 1026; Sulzberger, M., *Arch. Dermat. and Syph.*, 1929, **20**, 669; Nathan, E., and coworkers, *Klin. Wochenschr.*, 1929, **8**, 1714; Mayer, R. L., and Sulzberger, M., *Arch. f. Dermat. u. Syph.*, 1931, **163**, 245.

[3] Mayer, R. L., *Arch. f. Dermat. u. Syph.*, 1931, **163**, 223.

[4] Sulzberger, M., *Arch. Dermat. and Syph.*, 1930, **22**, footnote p. 849; Meyer, K., *Biochem. Z.*, 1925, **166**, 202; Bock, G., *Münch. Med. Wochenschr.*, 1929, **76**, 915.

[5] Kolle, W., *Reale Accademia d'Italia*, Convegno ''Volta,'' Rome, Sept. 25, 1933.

In a limited number of specificity tests with the substances named, in animals that gave marked homologous reactions there was, as a rule, no difficulty in recognizing reactions with the homologous compound by their greater intensity. The guinea pigs sensitive to p-phenylene diamine, in addition to the homologous reaction, gave a smaller but distinct reaction with nitroso-dimethylaniline.

Injections of aminoazobenzene, aminoazobenzene disulphonic acid, and the carbohydrate dextran have, so far, yielded negative results.

7321 P

Relationship Between Complement and Prothrombin.

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Bordet was the first to recognize a relationship between thrombin and complement. Fuchs[1] has recently claimed that the *mid-piece* of complement is identical with prothrombin. Since the complement content of plasma and serum are apparently the same, the change of prothrombin to thrombin does not seem to influence complement. A close association of prothrombin and thrombin with complement is indicated by:

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* The experiments with this substance were suggested to us by Prof. Zangger in Zürich. See Wedroff, N. S., *Arch. Gewerbepath. u. Gewerbehyg.*, 1932, **3**, 509.

[6] Landsteiner, K., and van der Scheer, J., *J. Exp. Med.*, 1932, **56**, 399.

[1] Fuchs, H. J., *Z. f. Immunitatsfor.*, 1929, **62**, 107.

TABLE I.

Volume of anticomplement	0.4	0.3	0.2	0.1	.09	.08	.07	.06	.05	.04	.03	.02
Serum heated to 56° C.	n.c.	c	c	c	—	—	—	—	—	—	—	—
Plasma ” ” ” ”	—	0+	+	++	+++	n.c.	c	c	—	—	—	—
Serum treated with $Mg(OH)_2$	—	—	—	0+	++	n.c.	c	c	—	—	—	—
Plasma ” ” ”	—	—	—	—	—	0	+	++	n.c.	c	c	c
Heparine (0.1% solution)	0	+	++	n.c.	c	c	—	—	—	—	—	—
Calcium chloride (M/10)	—	—	—	—	—	0	++	+++	n.c.	c	c	—

Each system consisted of 0.1 cc. of complement (1 unit)
0.4 cc. 2 x sensitized sheep cells
physiological saline **q.s. 1.0 cc.**

c — complete hemolysis
n.c. = nearly complete

A { 0.1 cc. oxalated plasma } clot in 105 sec.
{ 0.1 cc. calcium chloride (M/40) }
A + 0.05 cc. heated (56° C.) serum ” ” 120 ”
A + 0.05 cc. ” ” ” plasma ” ” 120 ”
A + 0.05 cc. serum treated with $Mg(OH)_2$ ” ” 135 ”

A + 0.05 cc. plasma treated with $Mg(OH)_2$ clot in 170 sec.
A + 0.10 cc. heparine (0.1% sol.) ” ” 180 ”
0.1 cc. plasma treated with $Al(OH)_3$ } ” ” 300 ”
0.1 cc. calcium chloride M/40 }

Plasma treated with $Al(OH)_3$ has the same content of complement as untreated plasma.

Treating serum or plasma with magnesium hydroxide or aluminium hydroxide consisted in mixing 0.5 cc. of the serum or plasma with 0.05 cc. of the reagent incubating at 37° C. for 15 min., and centrifuging.

1. Both complement and thrombin are non-specific. Complement obtained from one animal will react with the amboceptor and antigen of another species. Thrombin from various animals will clot fibrinogen irrespective of its source.
2. Both thrombin and complement, particularly the *mid-piece,* are associated with the globulin fraction of the serum proteins.
3. Prothrombin, thrombin, and complement are inactivated at 56°C., and the resulting serum or plasma becomes antithrombic and distinctly anticomplementary (the plasma more than the serum).
4. Serum or plasma treated with an emulsion of magnesium hydroxide becomes antithrombic (anti-clotting), and anticomplementary, with the effect more pronounced in the plasma than in the serum.
5. Heparine inactivates both complement and thrombin.
6. Calcium inhibits both complement and clotting. For the reactivation of prothrombin in oxalated plasma, there is an optimal concentration of calcium, below and above which clotting is retarded. Absence of calcium does not inhibit complement.

That prothrombin is not identical with complement is indicated by: 1. Alumina cream will remove prothrombin from plasma but will not alter the complement content. 2. Patients have been found whose plasma is anticomplementary, but have a normal clotting time.

Conclusion. Although a close association exists between prothrombin and complement, evidence is against the assumption that the former and the *mid-piece* of complement are identical.

7322 C

Globulin Extract of Immune Adult Serum in Prophylaxis of Measles.*

S. KARELITZ. (Introduced by Bela Schick.)

From Mount Sinai Hospital, New York City.

It is widely appreciated that the majority of infants up to 3 months of age and frequently longer possess immunity to certain diseases, such as measles, diphtheria, scarlet fever, and poliomyelitis. Explanations for these facts have been varied. Most of the experimental data, however, points to the transplacental transmission of antigen or antibodies as being the most acceptable explanation for the presence of immunity in the new born infants. De Souza and Jorge[1] believing that the placenta may produce non-specific immune substances, utilized cord blood in the prophylaxis of measles. Their studies made during the years 1929 to 1932 showed that with 4-10 cc. of this blood they obtained 67-100% protections in 146 infants and children exposed to measles in hospital wards. Four children exposed at home and treated prophylactically developed ordinary measles. Finkelstein[2] had excellent results using 40-60 cc. of puerperal serum, 84.5% protections in 148 children, against 79.2% with similar doses of convalescent serum in 311 children. Others[3] had less favorable results, using 20 cc. of cord blood.

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* This work was aided by a grant from Miss Emilie Fries.

McKann and Chu[4] believed that by extracting the placenta, substances were obtained which represented maternal blood, fetal blood, and probably extracts from the placental tissue itself, whereas blood obtained from the umbilical cord represented fetal blood only, and contained only those substances involved in immunity to which the placenta is permeable. Using the globulin extract of placenta for measles prophylaxis they obtained excellent results. Forty-three susceptible children, hospitalized for other conditions, and exposed to measles, were injected intramuscularly with placental extract. In no instances did the injected placental extract fail to prevent or modify the measles in the susceptible contacts.

It was our opinion that possibly the blood contained in the placenta was the source of this active globulin extract. If that were so this immune substance should be present not only in the mother's circulation, but in the circulation of any person immune to measles, facts known to be true. We therefore extracted the globulins from the blood serum of measles immune male adults and compared the prophylactic value of this extract with the serum from which it was obtained. No attempt was made to separate off the inactive euglobulin fraction from the active pseudoglobulin.

Forty-four children susceptible to measles, 41 ranging in age from 18 months to 8 years and 3 children 12 to 14 years were exposed to measles in their homes from 2-5 days. Each child was injected subcutaneously or intramuscularly in the thigh with 8 cc. of the serum obtained from either of 2 donors—R. and M. Of these 18, or 41%, remained completely protected, 17, or 9%, developed modified measles, and 9, or 20%, developed unaltered measles.

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[1] de Souza, J. S., *Arch. de Med. des Enfants*, 1932, **35**, 633.

[2] Finkelstein, G. S., *Vrach. Delo.*, 1931, **14**, 791; *J. A. M. A.*, 1932, **98**, 92.

[3] Dulitskiy, S. O., *J. rann. detsk. vozr.*, 1932, **12**, 322.

[4] McKann, C. F., and Chu, F. T., *J. Infect. Dis.*, 1933, **52**, 268. Also personal communication.

Some of the above serum was then precipitated with 50% ammonium sulphate and dialized to eliminate the ammonium sulphate. The globulin obtained was then preserved with a 1/7500 solution of merthiolate and diluted with normal saline solution so that 8 cc. of the solution represented the globulin extracted from 8 cc. of the original serum. The extraction was done by Mr. Klein in the laboratory of Dr. W. H. Park. It was found to be sterile.

Twenty-four susceptible children ranging in age from 2-14 years and exposed at home for 2-5 days each injected subcutaneously or intramuscularly with 8 cc. of the globulin solution, 12, or 50%, of these remained protected, 10, or 42%, developed modified measles, and 2, or 8%, failed to be helped. The latter 2 were sisters, 4 and 14 years of age, and had been exposed 7 or 8 days. The failures were therefore to have been expected. The results with the globulin extracts of sera R and M were almost identical with those of their original sera. Since some of the globulin was probably lost in the extraction, and since the average age of the children injected with the globulin was somewhat greater than with the control serum, it is possible that the globulin fraction was slightly more effective than the serum from which it was extracted.

The injection of the globulin extract intramuscularly or subcutaneously was associated with almost immediate severe local pain, burning or gnawing in character. The pain was frequently accompanied by headache, dizziness, facial pallor, and sometimes vomiting, reactions somewhat like those following adrenalin or pituitrin injections. The pain was very severe for 15-30 minutes and continued in milder form for several hours. It was in striking contrast to the mild local discomfort following the injection of the original serum.

McKann was able to get 20 to 30 effective prophylactic doses of globulin extract from each placenta. Assuming that under conditions such as those of our study, 8 cc. of immune adult blood serum, or about 20 cc. of whole blood is an effective prophylactic dose for measles, it would indicate that the placenta contains anti-measles substance equivalent to at least that which would be obtained from 20 times 20, or 400 cc. of blood.

Although the placenta is extremely rich in blood, it is questionable whether so large a percent of it is blood, and therefore doubtful whether the presence of the anti-measles substance can be explained on the blood-content alone. It is conceivable, however, that all of the placental fluid is comparable to blood serum and similar to it in its antibody content. It is questionable, therefore, whether it will be necessary to postulate other than humoral immunity transmitted from the mother to the placenta to explain the striking results recorded with placental globulin extract.

McKann's studies were nearly all done in hospital exposures, which according to the recent report of Schick and Karelitz,[5] usually yields a more optimistic or exaggerated impression of the actual value of a serum. If, therefore, the dosage of placental extract will have to be larger for children intimately exposed to measles, as is the case in their homes, it is possible that their results will more nearly approximate those obtained with blood of immune adults.

Conclusions. 1. The globulin of the blood of measles immune adults carries a factor effective in measles prophylaxis. 2. This globulin extract is at least as effective as the blood serum from which it was obtained. 3. Although no direct proof is offered, suggestions are made which might explain the striking results obtained with placental globulin extract, on the basis of maternal and fetal blood contained in the placenta.

7323 C

Effect of Type of Carbohydrate on Vitamins B and G Potency of Feces Voided by Rats.*

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In a previous publication from this laboratory[1] the danger of coprophagy, as it affects the assay of vitamins B and G, was discussed. Similar observations had been reported by Roscoe.[2] More recently Booher and Kaneko[3] have submitted data which led them to conclude that their assays for vitamin B (B_1) were not vitiated by coprophagy. The last mentioned investigators used raw corn

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[5] Karelitz, S., and Schick, B., *Wien. Med. Woch.*, 1932, **45**.

* Publication authorized by the Director of the Pennsylvania Agricultural Experiment Station February 21, 1934, as Technical Paper No. 637.

[1] Guerrant, N. B., and Dutcher, R. Adams, *J. Biol. Chem.*, 1932, **98**, 225.

[2] Roscoe, M. H., *Biochem. J.*, 1931, **25**, 2056.

[3] Booher, L. E., and Kaneko, T., PROC. SOC. EXP. BIOL. AND MED., 1932, **30**, 69.

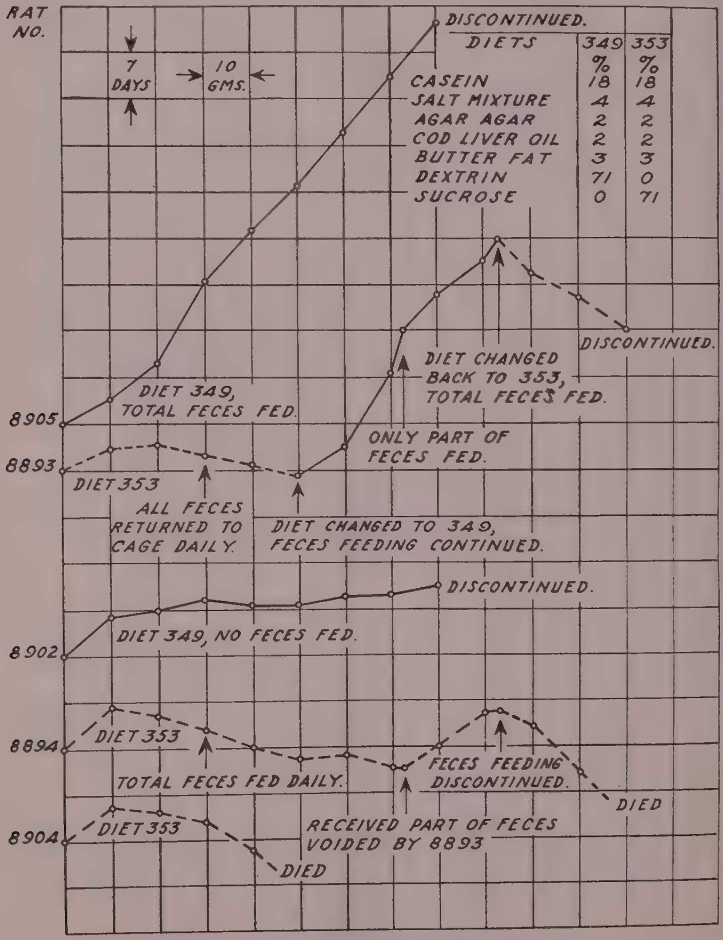
starch as the source of carbohydrate. Since Roscoe had reported that she was unable to obtain characteristic growth responses from coprophagous rats when raw corn starch served as the source of carbohydrate, it seemed highly probable that the type of carbohydrate might be an important factor worthy of further study. This possibility has been further suggested by data obtained in this laboratory during a somewhat related investigation in which it was found that the quantity of vitamins B and G required to produce a unit increment of growth was considerably greater when sucrose was fed as the source of carbohydrate than it was when dextrinized corn starch was used.

The experiments here described were initiated to determine the extent to which growth responses in coprophagous rats could be affected by substituting carbohydrates of various types in the usual basal ration deficient in vitamins B and G. Among the carbohydrates under investigation at present are: corn starch, dextrinized corn starch, glucose, sucrose, and lactose. While the results to date are insufficient for a complete statement, the differences obtained with diets containing sucrose and corresponding diets containing dextrinized corn starch are sufficiently marked to justify a preliminary note at this time.

The experimental technique employed was similar to that reported elsewhere.[1] The feces voided were removed from the cage-pans daily, placed in a separate feed container and returned to the cage of the animal which voided them or to that of another designated animal. The growth records of a few typical animals are given in Chart 1.

Animal 8904, weight 40 gm., received the sucrose-containing diet (353), unsupplemented. This animal made about the same increase in weight during the first week of the experiment that is usually observed when young animals receive this diet. During the remainder of the experimental period, there was a consistent loss in weight. The animal manifested its first paralytic symptoms on the 26th day and died on the 31st day.

Animal 8894, weight 43 gm., also received the sucrose-containing diet unsupplemented. The increase in body weight during the first week (8 gm.) was somewhat greater than that usually obtained while using this diet. During the following 2 weeks there was a gradual loss in weight. Beginning on the 22nd day, the feces of this animal were collected daily, placed in a separate food-container and returned to the cage. These feces were consumed quite readily during the first few days, after which the animal refused to ingest appreciable amounts. In the meantime the animal continued to lose weight and manifested slight paralytic symptoms. Starting on the 52nd day and continuing through the 66th day, this animal was given one-half of the feces voided daily by its litter mate (animal No. 8893), which at this time was receiving a dextrin-containing diet (349). Animal 8894 consumed these feces very greedily. During this 14-day period, the paralytic symptoms disappeared and the



DIETS	349	353
	%	%
CASEIN	18	18
SALT MIXTURE	4	4
AGAR AGAR	2	2
COD LIVER OIL	2	2
BUTTER FAT	3	3
DEXTRIN	71	0
SUCROSE	0	71

Chart I.

Showing typical growth responses made by young rats when fed vitamin B-complex deficient diets, unsupplemented and supplemented with feces voided by animals receiving the respective diets. The 2 diets differed in composition only in respect to the type of carbohydrate used.

animal increased 12 gm. in weight. On the 66th day feces feeding was discontinued and the animal lost weight gradually and died on the 81st day.

Animal 8902, weight 40 gm., received the dextrin-containing diet unsupplemented throughout the experimental period. During the first 4 weeks its deportment was quite comparable to that which is usually obtained on this diet. There were some indications, however, that during the last 4 weeks this animal was practicing a slight degree of coprophagy. This condition seems to be extremely difficult to avoid when the diet contains a relatively high percentage of dextrinized corn starch, especially when the experimental animal has been over-depleted of its vitamin B stores. This animal was removed from the experiment at the end of the eighth week.

Animal 8893 (litter mate of 8894), weight 40 gm., received the sucrose-containing diet (353), unsupplemented, during the first 3 weeks, and made a very typical growth response during this time. Starting with the 21st day, all feces voided by this animal were returned to the cage daily. From the 21st to the 35th day the animal continued to lose weight. On the 35th day the diet was changed from the sucrose diet to the dextrin diet, and the feces feeding was continued. During the next 17 days the weight of the animal increased 32 gm. Starting with the 52nd day and continuing for 2 weeks, only one-half of the feces voided by this animal was returned to the cage, the other half of the feces being fed to animal 8894 during this period. This animal (8893) continued to increase in weight so long as it received the dextrinized corn starch diet supplemented by its own feces. On the 66th day the diet was changed back to diet 353, and the feeding of all feces was continued from this date. Eighteen days later, the animal was removed from the experiment, after it had lost 19 gm. in weight.

Animal 8905 (litter mate of 8902 and 8904), weight 42 gm., received diet 349 throughout the feeding period. Starting with the second day of the experiment, all of the feces were returned to the cage daily. At first there was some hesitancy on the part of the animal to consume the entire supply of feces but this lack of appetite had completely disappeared by the fifteenth day. The animal made a total gain of 86 gm. during the 8 weeks' observation, and at the end of this time appeared to be normal in all respects.

Summary. Data are presented which indicate that the type of carbohydrate used in vitamin B and G deficient diets is an important factor in determining whether or not coprophagy will vitiate results when accurate assays of vitamins B and G are desired. Coprophagy appears to be of little consequence when sucrose is used as the sole source of carbohydrate, while dextrinized corn starch offers the possibility of introducing serious errors and incorrect conclusions if rats resort to coprophagy when this carbohydrate is used.

Experiments are now in progress in which an attempt is being made to find an explanation for the results described, and the study is being extended to include other types of carbohydrates.

7324 C

Death of a Yeast Culture, as Registered by the Electric Resistance.

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The method used for estimating the vitality of tissues by measuring their electric resistance has been applied, in the present investigation, to a suspension of yeast heated gradually until all the cells were killed.

A suspension of 3 gm. of yeast (Fleischmann's yeast) in 3 gm. of a N/5 solution of NaCl, was heated from 15°C. to 93° and then cooled to 15° in an Ostwald's conductivity cell immersed in a water bath, and the electric resistance of the suspension was taken at each degree. The time between 2 readings was one minute. A microphone Hummer, working on a 6 volts battery, delivered the alternating current of 1000 cycles, used in the conductivity bridge. The suspension was stirred before each measurement by moving the electrodes up and down. Previous tests showed that the concentration N/5 did not affect the resistance of the cells to an appreciable amount.

The results, plotted on the curve (Fig. 1), show:

1. From 15° to 62° and from about 60° to 15° the resistance behaves like the resistance of a salt solution, and the curve coincides with the resistance curve of a salt solution of convenient concentration.

2. From 62° to 67° the resistance falls abruptly. This irreversible drop is probably due to the death of the majority of the cells.

3. From 67° to 93° and from 93° to about 60° the curve shows a resistance higher than would be expected from a salt solution; at about 60° it coincides again with the typical curve of such a solution. This phenomenon suggests that above 67° the few surviving cells are killed.

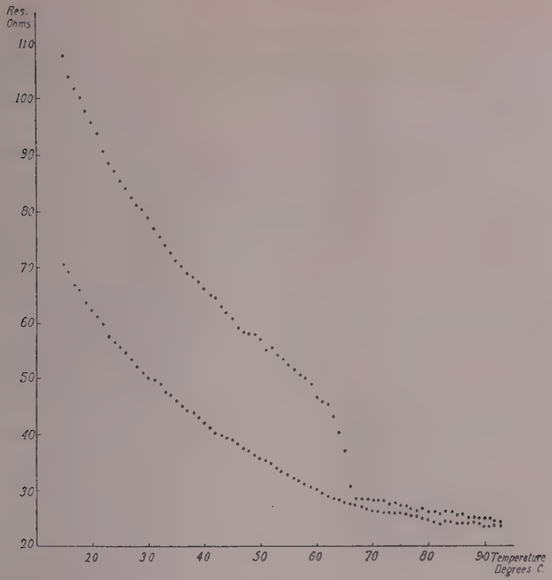

FIG. I.

Specific resistance of the yeast suspension when heated from 15° to 93° (upper curve) and when cooled from 93° to 15° (lower curve).

Yeast subjected to temperatures ranging from 40° to 95°, at intervals of 5°, during 5 minutes for each interval, and then cultured on agar plates, gave a gradually decreasing growth for temperatures extending from 50° to 75°, but traces of growth were found in specimens heated at 80°, 85° and 90°.

A 2% eosin solution did not penetrate the cells when the latter were exposed to temperatures lower than 55°. In suspensions heated at 55°, 60°, 65°, and 70° for the length of time above mentioned, the number of stained cells increased gradually. After treatment at 75° and above, all the cells took the stain readily.

The drop of resistance seems to delimitate the extension of the lethal effect of heat in yeast cells more sharply than any other indicator of death.

7325 C

Effects of Prolonged Viosterol Administration and of Artificial Sunlight Irradiation on Experimental Tuberculosis in Rabbits.*

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At the 1932 meeting of the National Tuberculosis Association, we reported favorable results from the treatment of tuberculous guinea pigs with viosterol and ascending doses of tuberculin.[1] In the present work a similar though more extensive investigation was performed upon albino rabbits. In addition to the viosterol-treated animals, certain groups were treated with artificial sunlight irradiation to determine whether the irradiation might not have an effect similar to that produced by viosterol.

The rabbits were inoculated subcutaneously with a lethal dose of bovine tubercle bacilli (0.01 mg. of a culture, 0.001 mg. of which was known to produce tuberculosis in the rabbit) and were divided into 6 groups of 8 animals each. The respective groups received treatment as follows: I, viosterol; II, viosterol plus tuberculin; III, ultraviolet irradiation and maize oil; IV, ultraviolet irradiation and maize oil plus tuberculin; V (tuberculin controls), tuberculin and maize oil; VI (untreated controls), maize oil. The amounts of maize oil given to the groups that did not receive viosterol were in every case equal to those given to groups I and II as a medium for the viosterol.

Treatment was started 21 days after the inoculation. The original 10,000 X viosterol solution† was diluted with maize oil to contain 1.46 mg. in 0.05 cc.; this amount per pound of body weight was given weekly by intramuscular injection. Tuberculin (bovine, O.T.) was given subcutaneously simultaneously with the viosterol in weekly ascending doses from 0.00001 mg. to 18.5 mg. for 34 consecutive weeks. Irradiation was given every second day. For the source of light a carbon arc lamp drawing 66 amperes with 48.5 volts across the arc was used. The intensity of radiation of visible and ultraviolet light from the arc was equal to 80 watts per square meter at a distance of one meter with 38% of this coming from rays shorter than 4000 Ångstrom units. The initial dose for the irradiation was 2 minutes at a distance of one meter from the arc. This was gradually increased to 12.5 minutes at 0.7 meter on the forty-second treatment; after this, the dose remained constant and was sufficient to cause a definite erythema of the rabbits' ears. In the combined irradiation and tuberculin group, irradiation was given every second day; tuberculin, in weekly doses. At the death of an animal, autopsy findings were recorded and tissues were examined histologically. During the 34th week, the surviving animals were sacrificed.

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* A more detailed report of this work was submitted by Miss Trevorrow as a thesis to the University of Colorado, August, 1933, in partial fulfillment of the requirements for the M.S. degree.

[1] de Savitsch, E. C., Trevorrow, V. E., Black, W. C., and Lewis, R. C., *Tr. Nat. Tuberc. Assoc.*, 28th annual meeting, 1932, 187; *Am. Rev. Tuberc.*, 1933, **28**, 699.

†Furnished through the courtesy of Mead Johnson and Co.

The results obtained indicate that both prolonged administration of viosterol and irradiation markedly increase the tendency toward central calcification of necrotic tubercles. The incidence of such calcification was most marked (92 to 94%) in the groups that were treated with viosterol alone, with viosterol and tuberculin, and with irradiation and tuberculin, respectively, and somewhat less so (60%) when irradiation without tuberculin was used. These figures are in contrast to an incidence of 25% calcification of tubercles for the tuberculin controls, and of 14% for the untreated controls. However, the degree of organ involvement was greater, and the survival period less, in the animals that received treatment with viosterol or by irradiation than in those of the control groups. When tuberculin was used in conjunction with either of the other treatments, this detrimental effect was definitely less pronounced, but still evident.

The average figures for the survival period of each group are: I (viosterol) 165 days; II (viosterol plus tuberculin) 183 days; III (ultra-violet irradiation and maize oil) 190 days; IV (ultraviolet irradiation and maize oil plus tuberculin) 222 days; V (tuberculin and maize oil) 266 days; VI (maize oil) 258 days.

Our failure to obtain a positive therapeutic effect from the viosterol-tuberculin treatment in rabbits similar to that observed by us[1] in guinea pigs cannot readily be explained, but the beneficial results reported by de Savitsch, Stewart, Hanson, and Walsh[2] in tuberculous rabbits similarly treated for a shorter period of time suggest that the larger total amount of viosterol used by us may have proved detrimental on account of its toxicity to the rabbit.

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[2] de Savitsch, E. C., Stewart, J. D., Hanson, L., and Walsh, E. N., *Tr. Nat. Tuberc. Assoc.*, 29th Annual Meeting, 1933, 216.

7326 C

Liebermann-Burchard Reaction with Carotene.

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We have postulated some common chemical configuration, actually present or easily produced as a result of chemical interaction, in sterols, in carotene, in the fat-soluble vitamins A, D, and E, in terpenes, and in 5-membered monoheterocyclic compounds.[1] We have recently demonstrated that a mixture of formaldehyde and concentrated sulphuric acid constituted a very sensitive reagent for the detection of carotene in chloroform solution.[2] Whitby,[3] employing the same reagent, reported for cholesterol a color reaction which, however, is not identical with the one obtained for carotene. In this communication we report our findings with reference to the Liebermann-Burchard reaction with carotene. The reaction is the familiar one used in the qualitative detection and in the quantitative estimation of cholesterol. We have already shown that this reagent reacts with heterocyclic compounds like thiophene, furfurane and derivatives.[4]

Carotene gives a positive response with the Liebermann-Burchard reagent, using 10 cc. of chloroform solution, 2 cc. of acetic anhydride and 0.2 cc. concentrated sulphuric acid. With low concentrations of carotene a green color is obtained, which matches in color the one developed when cholesterol is treated with the same reagent. In higher concentrations the green color is tinged faintly blue, but can still be matched with the color obtained with cholesterol. Copper-colored crystals of carotene exposed to sunlight and air turn yellow brown. Carotene, thus oxidized, does not give the characteristic color when dissolved in chloroform and treated with acetic anhydride and sulphuric acid. A brown color instead of the green develops. Old solutions of carotene in chloroform also react in the negative. Baumann and Steenbock[5] have recently shown that carotene in organic solvents deteriorates on standing. We therefore used freshly prepared chloroform solutions of carotene for our tests.

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[1] Levine, V. E., and Richman, E., *J. Biol. Chem.*, 1933, **101**, 373; *Biochem. J.*, 1933, **27**, No. 6, 2051; Levine, V. E., and Seaman, C. L., *Biochem. J.*, 1933, **27**, No. 6, 2047; Levine, V. E., and Shaughnessy, E. J., *Biochem. J.*, 1933, No. 6, **27**, 2048.

[2] Levine, V. E., and Bien, G. E., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 581.

[3] Whitby, G. S., *Biochem. J.*, 1923, **17**, 5.

[4] Levine, V. E., and Richman, E., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 582.

The color obtained with carotene reaches its maximum intensity immediately and fades within 2 minutes, leaving a dirty yellow brown color. The green color obtained with cholesterol develops but slowly, reaching its maximum intensity within 15 minutes. The limit of the sensitivity for carotene is 0.01 mg. in 10 cc. of chloroform. The limit of sensitivity for cholesterol is 0.01 mg. in 10 cc. of chloroform as judged with the naked eye and 0.04 mg. in 10 cc. of chloroform as judged in the colorimeter. No attempt was made to determine the sensitivity of carotene with reference to a colorimetric reading owing to the rapid fading of the color.

The Liebermann-Burchard reaction evidently is not given by sterols alone, but also by carotene and certain 5-membered monoheterocyclic compounds. In addition, caryophillin, urson (ursolic acid), rosin, rosin acids, and rosin oil have been reported to give positive tests.[6]

Pure cholesterol in chloroform solution yields higher figures with the Liebermann-Burchard quantitative procedure when carotene is added.

TABLE I.
Effect of Carotene on Cholesterol Determinations.

Reading after	Mg. Cholesterol in terms of 100 cc. of blood	% error
Series I. 0.72 mg. cholesterol + 0.2 mg. carotene in 10 cc. of chloroform, corresponding in the Myers and Wardell[7] method for cholesterol to 180 mg. of the sterol and 50 mg. carotene in 100 cc. of blood.		
8 min.	218	21.10
10 "	208	15.55
12 "	205	13.88
15 "	198	10.00
Series II. 0.72 mg. cholesterol + 0.1 mg. carotene in 10 cc. of chloroform, corresponding to 180 mg. cholesterol and 25 mg. carotene in 100 cc. of blood.		
8 min.	202	11.22
10 "	201	11.66
12 "	194	7.78
15 "	191	6.11
Series III. 0.72 mg. cholesterol + 0.05 mg. carotene in 10 cc. of chloroform, corresponding to 180 mg. cholesterol and 12.5 mg. carotene in 100 cc. of blood.		
8 min.	202	12.22
10 "	200	11.11
12 "	194	7.38
15 "	186	3.33

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[5] Baumann, C. A., and Steenbock, H., *J. Biol. Chem.*, 1933, **101**, 561.

[6] Honeywell, E. M., and Bills, C. E., *J. Biol. Chem.*, 1933, **103**, 515; Sando, C. E., *J. Biol. Chem.*, 1923, **56**, 464; Dodge, F. D., *J. Am. Chem. Soc.*, 1918, **40**, 1931; Wiley, H. W., and Bigelow, W. D., *U. S. Dept. Agr. Bur. of Chem. Bull.*, **65**, 1902.

[7] Myers, V. C., and Wardell, E. L., *J. Biol. Chem.*, 1918, **36**, 147.

The error due to carotene diminished with time. This fact is important since in the cholesterol determination of blood the colorimetric reading is made 15 minutes after the addition of acetic anhydride and sulphuric acid to the chloroform extract.

In the method for blood cholesterol the blood is taken up with plaster of Paris. This is dried, pulverized, and extracted with chloroform. The plaster of Paris retains the carotene. The pure pigment, however, is not adsorbed by plaster of Paris. Carotene was added to a chloroform solution of cholesterol, incorporated with plaster of Paris, and the organic solvent allowed to evaporate. The dried mixture was extracted from 1 to 2 hours with chloroform in a Soxhlet extractor. The chloroform solution assumed the color characteristic of carotene in this solvent. As the heating and extraction proceeded the chloroform turned lighter and finally assumed a faint yellow tint. The change in color is probably due to oxidation. The faintly colored chloroform solution gave but a slight green with the Liebermann-Burchard procedure.

Carotene added to blood in the proportion of 100 mg. per 100 cc. and treated with plaster of Paris as in the Myers and Wardell procedure for cholesterol did not increase the original cholesterol value of the blood. This quantity of carotene is extremely high and is hardly to be expected under any physiological or pathological condition. White and Gordon[8] reported in 18 normal human beings a mean value of 0.063 mg. of carotene per 100 cc. of serum, the highest value being 0.108 mg. In 14 diabetics they found a mean value of 0.213 mg. in 100 cc. of serum, the highest figure being 0.379 mg. Curtis and Kleinschmidt[9] reported a case of carotenemia in which the carotene content at the height of the condition was 1.65 per mg. 100 cc. of serum.

Carotene seems to be easily adsorbed. Palmer[10] demonstrated that carotene cannot be determined in whole blood due to the interference of the erythrocytes and in serum only when serum proteins are removed. A mixture of serum and plaster of Paris must be treated with alcohol to precipitate the proteins and to release the carotene preliminary to extraction with petroleum ether.

Crampton and Simons[11] in 1905 applied the Liebermann-Storch reaction for rosin oil for the detection of palm oil used in coloring other fats and oils. Their method consisted in developing a blue color with a faint green tint in a mixture of equal parts of melted fat or oil and of acetic anhydride to which a drop of concentrated sulphuric acid was subsequently added. Chloroform was not used as a solvent for the fat or oil. Bryan and Gardner[12] maintained that the test was not specific, since other oils responded, that the color faded rapidly and varied greatly with the quantity of concentrated sulphuric acid used. Gill[13] reported that the test for palm oil as made by Crampton and Simons was due to the presence of carotene. He obtained positive spectographic reactions for carotene in carbon disulphide extracts of the non-saponifiable residue secured from palm oil and from the fat of orange peel, yellow corn, yellow squash, flax seed, mustard seed and black sesame seed. These extracts also gave positive tests with the Crampton and Simons procedure.

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[8] White, F. D., and Gordon, E. M., *J. Lab and Clin. Med.*, 1931, **32**, 17, 53.

[9] Curtis, A. G., and Kleinschmidt, E., *Ann. Int. Med.*, 1932, **6**, 751.

[10] Palmer, L. S., Carotinoids and Related Pigments. The Chemical Catalogue Co., N. Y., 1922, p. 207.

[11] Crampton, C. A., and Simons, F. D., *J. Am. Chem. Soc.*, 1905, **27**, 270.

Employing the Liebermann-Burchard method of testing, we examined several oils, 5 drops of which were dissolved in 5 cc. of chloroform. Our samples of linseed oil and sesame oil gave a dark brown or mahogany color. Cod liver oil yielded a green, which became greenish brown and finally changed to brown. Cotton seed oil, olive oil, castor oil, and wheat germ oil responded with the characteristic green color. Evanescence of color was taken to indicate the presence of carotene rather than cholesterol. Crampton and Simons reported positive color reactions with sesame oil and mustard oil, and Gill noted a positive reaction with linseed oil. The results we obtained with our samples of sesame oil and linseed oil do not agree with those of Gill and Crampton and Simons, but indicate, however, the presence of oxidized carotene, since we obtained a dark brown color instead of the characteristic green color. McDonald[14] and Baumann and Steenbock have recently reported that carotene in solution in various oils oxidizes more or less rapidly on standing as a result of the action of light and air.

Conclusions. Carotene can readily be detected in as low a quantity as 0.01 mg. dissolved in chloroform by means of acetic anhydride and concentrated sulphuric acid. The reaction is similar in method of performance and in final color production to the one employed in the Liebermann-Burchard test for cholesterol. The difference in the duration of the green color developed may serve, however, to differentiate one compound from the other. The green color obtained with carotene develops its maximum intensity at once and fades within 2 minutes, leaving a dirty yellow brown color. The green color obtained with cholesterol develops its maximum intensity at the end of 15 minutes.

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[12] Bryan, T. J., and Gardner, B. C., *U. S. Dept. Agr., Bur. Chem. Bull.*, **137**. 1911, 87.

[13] Gill, A. H., *J. Ind. Eng. Chem.*, 1917, **9**, 136; 1918, **10**, 612.

[14] McDonald, F. C., *J. Biol. Chem.*, 1933, **103**, 455.

Carotene does not interfere with the quantitative estimation of cholesterol in the blood. The extraction with chloroform of a mixture of blood and plaster of Paris removes the cholesterol, but does not release the carotene present.

7327 P

Immunization of Guinea Pigs with Formalized Cultures of European Strain of Typhus Rickettsia.

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We have reported[1] the successful cultivation of the European strain of Typhus *rickettsia* in flask cultures containing guinea pig tunica. These cultures have now passed successfully through 7 subcultures during a period of 4 months.

In view of successful immunization of guinea pigs with formalized rickettsia suspensions from infected lice[2] and Zinsser's[3] experience with formalized rickettsia suspensions of the Mexican type from infected X-rayed rats, it appeared desirable to test the possibility of using flask tissue cultures for this purpose. Our efforts to immunize guinea pigs with formalized infected brain tissue proved unsatisfactory. Dead virus produced at best only a slight degree of immunity, when large amounts (10 gm.) of formalized infected tissue were injected. Solid immunity could only be obtained with tissue formalized for 1-2 hours, that is tissue still containing live (attenuated?) virus. The difficulty seems to lie in the quantity of organisms, or in other words the amount of antigen, injected. The cultures appeared to offer a satisfactory solution to the problem. On the one hand, the organisms are present in fairly large quantities and, on the other, the cultures are more easily handled than lice.

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[1] Kligler, I. J., and Aschner, M., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 349.

[2] Kligler, I. J., Olitzki, L., and Aschner, M., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 456.

[3] Zinsser, H., and Castaneda, M. R., *J. Exp. Med.*, 1933, **57**, 381.

Guinea pigs have been successfully immunized with formalized suspensions of fresh virulent cultures as well as with old cultures no longer infective for guinea pigs.

Experiment 1. The material from 2 flasks of an 11-day-old virulent culture, second generation, was mixed, centrifuged, the supernatant fluid decanted, the sediment thoroughly triturated in a glass mortar and resuspended in the clear supernatant fluid. A formalin solution was added to the suspension to give a 0.1% concentration and the material left overnight at ice box temperature. Each of 2 guinea pigs was given 3 injections intraperitoneally of 1.5 cc. of the diluted formalized material, at intervals of 3 days. Each animal received the material of one culture. None of the animals developed any sign of fever or even a slight rise in temperature. The temperature during the immunization did not exceed 39°C., that is, it remained within the normal range of temperature of our guinea pigs.

Ten days after the last injection the guinea pigs received 80 infective doses and 16 days later 800 infective doses of brain virus. Neither manifested temperature or any other reaction. Appropriate controls came down with typical infections.

Experiment 2. In this experiment a series of old cultures, 30 to 55 days old, 4th to 6th generation, was used. They apparently no longer contained viable organisms since 1 cc. of a heavy suspension of the triturated tissue failed to infect a guinea pig. These cultures were treated as above and 4 guinea pigs immunized by 3 injections of 2 cc. each, at intervals of 3 days. Tested 6 and 18 days after the last injection all 4 guinea pigs proved immune to 80 and 800 infective doses of brain virus.

It is apparent, therefore, that formalized flask tissue cultures of the European strain of Typhus *rickettsia* provide as effective a vaccine for guinea pigs as do formalized *rickettsia* suspensions from infected lice. These cultures offer a relatively simple procedure for the production of vaccine *in vitro,* since old cultures produce as effective immunity as fresh cultures.

7328 P

Nerve Pattern in Regenerated Urodele Limbs.

PAUL WEISS AND ROLAND WALKER. (Introduced by R. G. Harrison.)

From the Osborn Zoological Laboratory, Yale University.

The factors determining the course, grouping, distribution, and size of peripheral nerves in development are still very much in the dark. One way of investigating these factors is direct experimentation on the embryo (Harrison,[1] Braus[2]). Another way is to study the development of nerves in regenerating limbs of adult urodele Amphibians. Limb regeneration in these animals is known to be a sort of second development (P. Weiss), differing from the first development in the mass of tissue involved, the time relations, the conditions of muscle differentiation and of nerve supply. A comparison of nerve patterns established during regeneration with those formed during embryogenesis can be expected to yield some clue as to which factors may, and which may not be significant. If merely the nerves supplying a limb are severed, nerve regeneration into the limb takes place from the proximal stumps, the outgrowing fibers mainly following the old degenerated peripheral pathways which have persisted as such. If the whole limb is amputated, differentiation of a new limb occurs out of an undifferentiated blastema in which no old pathways are present to guide the outgrowing nerve fibers. The question arises as to what sort of pattern will be assumed by the nerves in the regenerating limb.

In metamorphosed individuals of the newt, *Eurycea bislineata* (Green), of varying age and size, the left fore limb and the right hind limb were amputated at their bases. The limb nerves were cut at about the level of the girdle plexus. Eight animals were kept for from 176 to 202 days, and were then preserved for dissection. Regeneration had taken place in all of the hind limbs and in 5 of the fore limbs. The regenerates varied from one-half to three-quarters the length of controls on the opposite side. They were slenderer in proportion but otherwise well formed. Some of the animals died before observations were made on the function of the limbs, but in all those observed there was normal coordination of movements. In one case the extension of the elbow was somewhat impaired.

All 4 limbs were dissected, the distribution of nerves in the regenerated limb being compared with that of its mate on the opposite side. It was assumed that there would normally be less variation between the members of a pair of limbs than between the homologous limbs of different individuals. Attention was directed chiefly toward the plexuses, the nerves of the upper arm, and the sciatic and its ramifications as far as the knee. The results were as follows:

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[1] Harrison, R. G., *J. Exp. Zool.*, 1907, 4, 239.

[2] Braus, H., *Anatom. Anzeiger*, 1905, **26**, 433.

The main muscle masses of the arm and thigh are essentially normally arranged in the regenerating limbs, though minor aberrations of subdivision or attachment are found, and in some cases there are notable deficiencies in relative volume. In general the nerves keep a normal spatial relation to these muscle masses and to the skeleton. For instance, the superior (radial) and inferior (ulnar) long brachial nerves always enter the arm next to each other between the heads of the anconaeus, and then reach the flexor surface of the forearm by their usual opposite spirals around the humerus, the radial constantly lying between the 2 more lateral heads of the anconaeus, and the ulnar running deep to the anconaeus coracoideus and the coraco-brachialis longus. In the thigh the sciatic and most of its branches always run in the space between the femur and the flexor and extensor masses, covered superficially by the m.ilio-fibularis. The points where the minor branches leave the main trunks are variable, but scarcely more so than in the normal limbs of different individuals, and each muscle group seems always to be innervated by the usual nerve. There is some plexus formation between, and subdivision of the nerves along their course in the free limb, especially in the thigh. Such plexus formation is also present in the control limbs, but there is significantly more of it in the regenerates. In direct comparison of the two sides, there is a range of cases from very close symmetry of the two limbs, to very irregular plexus formation in the regenerated limb. Even in the latter cases, however, the main course of the nerve trunks is the same on both sides.

One striking point is the close correlation between the volume of the muscles in the regenerated limb and the diameter of the nerves innervating them. The nerves proximal to the level of amputation, however, show no reduction. The extreme of this condition is found in those cases where the fore limb had entirely failed to regenerate. Here the trunks of the 3rd and 4th spinal nerves of the two sides correspond exactly in diameter and position, but on the operated side stop short at the level of the plexus, giving off only a few branches to the girdle muscles.

It would seem that there are factors present in the regenerating limb which lead to the growth and grouping of entering nerve fibers along essentially typical major pathways, and to their distribution to the muscles according to typical spatial relations.

7329

Reaction of Anterior Pituitaries of Immature and Mature Female Rats to Injection of Pregnancy Urine Extracts.

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It has been previously reported[1] that injections of an oestrin-free placenta extract, containing the A. P. L. factor, into immature female rats induced a marked increase in the weight of the ovaries and a moderate increase in the weight of the pituitaries. Histologically, the pituitaries of the injected rats showed a slight to moderate decrease in the level of the eosinophiles; a considerable number showed evidence of granular loss. The most evident changes were found in the basophiles, which showed marked evidence of granular depletion.

Injection of pregnancy urine extracts into immature female rats gives similar results. Thirty rats, 21 to 25 days old, were given daily injections of pregnancy urine extract† (25 to 75 R. U. daily for 6 to 15 days). Twenty-eight littermate sisters served as controls. The ovaries of the injected rats weighed from 50 to 80 mg., while those of the controls weighed from 15 to 25 mg. The pituitaries of the injected animals were usually, but not invariably, increased in weight. Complete serial sections of all pituitaries were cut, at 2 micra, in the horizontal plane. One section from each ribbon of 15 was mounted and stained. Cell counts were made on 5 sections, equidistant apart from each set of serial sections (110,039 cells were counted on the 208 sections studied). The anterior lobes of the injected animals were definitely altered from those of the

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* This work has been supported by grants from the Committee for Research on Problems of Sex of the National Research Council, from the Committee on Scientific Research of the American Medical Association, and from the Division of Medical Sciences of the Rockefeller Foundation.

[1] Wolfe, J. M., Phelps, Doris, and Cleveland, Rucker, PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1092.

† Follutein was furnished by E. R. Squibb & Sons through the courtesy of Dr. J. J. Durrett.

controls. The eosinophiles were almost invariably reduced in number, and many of them were swollen and showed granular loss. The most marked changes were found in the basophiles, which showed reduction of granules. There was an increase in the percentage of the chromophobes. Mitoses were found in the chromophobes and eosinophiles of both the injected and control animals; they were more abundant in the injected animals. Mitotic division has never been observed in the basophiles.

We have pointed out[2, 3] that injections of extracts of pregnancy urine into mature female rats brought about marked changes in the anterior hypophysis, as well as in the ovaries, but such injections had no effect on the pituitaries of castrated female rats. This finding has also been reported by Severinghaus.[4] In our work, carried out on 90 injected and 153 control animals, it was found that pregnancy urine extract (75 R. U. daily for 15 days) produced a marked increase in the weight of the ovaries (106 to 765 mg.) and the pituitaries (8.5 to 26 mg.) of the normal females. The pituitaries showed loss of granules from both the eosinophiles and the basophiles; this was most marked in case of the basophiles. The levels of both of these cell types was reduced, while that of the chromophobes was increased. As pointed out before, this was an exaggeration of the changes found in early pregnancy or pseudopregnancy.[5] Mitoses were abundant in the chromophobes and to a lesser extent in the eosinophiles. Colloid was found in the cleft in nearly all animals.

The ovaries and pituitaries of 6 rats, receiving 25 units of pregnancy urine extract daily, for 7 weeks, were altered from those of rats receiving injections for only 15 days. The ovaries were normal or slightly above in size, confirming Collip, Selye and Thompson.[6] The pituitaries were also normal in weight, the pituitary weight paralleled the ovary weight. Histologically, the pituitaries showed a normal level of eosinophiles. All were well packed with granular material (compare with pituitaries of animals injected for 15 days). However, the level of the basophiles was still low, those present showing extreme granular depletion. No mitoses were present.

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[2] Wolfe, J. M., Ellison, E. T., and Rosenfeld, Louis, *Anat. Record*, 1934, **58**, 93.

[3] Wolfe, J. M., Ellison, E. T., and Rosenfeld, Louis, *Anat. Record*, 1934, **58**, 94.

[4] Severinghaus, A. E., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 593.

[5] Wolfe, J. M., and Cleveland, Rucker, *Anat. Record*, 1933, **56**, 33.

[6] Selye, H., Collip, J. B., and Thomson, D. L., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 566.

The above studies indicate that injections of extracts of human pregnancy urine induce marked changes in the pituitaries of immature and mature female rats, if the ovaries are present. Changes occur in both the basophiles and the eosinophiles, particularly notable was a loss of granular material. This is usually extreme in the basophiles and more or less marked in the eosinophiles. (The eosinophiles show granular loss only when active corpora lutea have been stimulated in the ovaries.) The granular cells, being depleted of granules, become chromophobes. Such loss of granules from either the eosinophiles or basophiles (or both) has been considered indicative of an active secretory state.

7330 C

Effects of Fat-Free Diet on Histological Fats in Various Organs of the Rat.

H. G. RICE AND C. M. JACKSON.

From the Department of Anatomy, University of Minnesota.

It has been shown[1] that rigid exclusion of fat from the diet of the rat induces a characteristic deficiency disorder, since the animal is apparently unable to synthesize certain fats which are necessary. The present study was undertaken to determine whether the histological fat distribution in the various organs is affected in this disorder. The organs studied include the liver, kidney, trachea, lung, skin and tela subcutanea.

Through the courtesy of Doctors Burr and Brown, 26 typical test rats that had been maintained several months on the fatless diet (No. 550B, plus vitamin supplements) were available, together with 5 rats "cured" by the subsequent addition of the necessary fats to the diet. These rats were killed by chloroform and autopsied in the usual way. The fats were studied chiefly in formalin-fixed, frozen sections stained with a solution of "oil red O" in diacetin (which presumably stains all fats and lipoids), though in some cases other methods were used. The technique is explained in detail in a separate paper dealing with the normal distribution of fats at various postnatal stages in the rat.[2] Both test rats and controls were females of the Wistar strain.

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[1] Burr, G. O., and Burr, M. M., *J. Biol. Chem.*, 1929, **82**, 345; 1930, **86**, 587. Burr, G. O., and Brown, W. R., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1349.

[2] Rice, H. G., and Jackson, C. M., *Anat. Rec.*, in press.

For comparison, 3 types of controls were used. (1) Eleven *direct controls* were reared on the same fatless diet (550B) with the addition of the necessary vitamins and fat. This is a high carbohydrate diet, containing 84% sucrose. (2) Eight *stock diet controls* were reared on our normal colony diet (McCollum's diet I), containing 67.5% ground wheat, 15% casein, 10% whole milk powder, and 5.2% butter-fat. (3) Since the test rats were retarded in growth and somewhat emaciated, 5 *inanition controls* were held by simple underfeeding of the stock diet at a body weight corresponding to that of the test rats.

The histological appearance of the fats and lipoids in the various organs of the normal rat (on both normal stock and high carbohydrate diets) is described elsewhere.[2] In general, we find that the trachea, lung and skin, aside from individual variations, show essentially the same amount, character and distribution of fats in all the groups studied, both test rats and controls. The *tela subcutanea,* however, is definitely atrophic in the test rats and inanition controls. This is probably due to the general state of undernourishment, producing moderate emaciation in these 2 groups.

In the liver, the test rats show a variable amount of fat in both glandular and Kupffer cells. The fat appears as fine granules (liposomes), and often also as coarser droplets especially in the periphery of the hepatic lobules. In some cases the hepatic fat in the test rats does not exceed that in the stock diet controls; in others the fat is abundant and definitely increased. The liver in the "cured" group usually shows a similar increase of fats and lipoids. In the direct controls (on high carbohydrate diet) there is likewise a tendency to an increased amount of visible hepatic fat, in agreement with the observations of Jackson.[3] Also in the inanition controls there is more hepatic fat than in normal stock controls, which were fully fed on the same diet. Jackson[4] reviewed the literature showing that hepatic steatosis may occur under various dietary conditions, including overfeeding as well as inanition of different types.

The kidney is of especial interest as the site of characteristic lesions in the fat-deficiency disorder.[5] In the test rats the amount of fat in the renal cortex varies from scanty to abundant. It may occur as liposomes in the cells of the convoluted tubules and, to some extent, in the interstitial tissue. In the renal medulla the fat is similarly variable, but with a tendency to greater abundance, especially in the interstitial tissue. Liposomes of variable size may occur in the epithelium of the papillary ducts and surface of the pyramids. They are also very frequent in the epithelium of the loop of Henle, which also often contains fatty casts. Borland and Jackson[5] thought that the fat in these casts is probably excreted through the convoluted tubules. There is no evidence for a glomerular origin.

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[3] **Jackson, C. M., *J. Nutrition,* 1930, 3, 61.**

[4] **Jackson, C. M., *The Effects of Inanition and Malnutrition upon Growth and Structure,* Philadelphia, 1925.**

[5] **Borland, V. G., and Jackson, C. M., *Arch. Path.,* 1931, 11, 687.**

In the direct controls (on high carbohydrate diet) as well as the "cured" group the amount of renal fat is likewise usually somewhat increased in comparison with that found in the normal stock diet controls. This is in agreement with Borland and Jackson.[5] Wegelin[6] also found increased fat in the renal epithelium and interstitial tissue of animals on high carbohydrate or fatty diets. He interpreted this as evidence of renal secretion of excess fat. The inanition controls of the present series likewise show a tendency toward increased renal fat, which is a typical effect of inanition.[4]

In summary, the test rats on fatless diet show no apparent change in the amount, character and distribution of the visible (stainable) fats in the trachea, lung and skin. The decrease of fat in the tela subcutanea is probably due to the moderate emaciation of the test rats. The frequent increase of fats and lipoids in the liver and kidney may be ascribed to the effect of 2 factors—the high carbohydrate diet and the state of general undernourishment. It may, therefore, be concluded that the fatless diet apparently produces no *specific* changes in the visible fats and lipoids of the organs examined.

7331 C

Factors which Determine Renal Weight. XVII. Influence of Age.

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We have concluded (MacKay and MacKay[1]) that in the albino rat the kidney weight has practically the same relation to body surface at all ages. In a broad sense this is true. However, a close inspection of the data presented at that time, omitting the less reliable figures obtained after 400 days of age, shows a slight but definite fall in the kidney weight: body surface ratio with increasing age. It has seemed desirable to call attention to this fact and to point out the cause.

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[6] Wegelin, C., *Berl. klin. Wchnschr.*, 1913, **50**, 2125; 2190.

[1] MacKay, L. L., and MacKay, E. M., *Am. J. Physiol.*, 1927, **83**, 191.

Attention has been drawn (MacKay and MacKay[2]) to the decrease in the caloric intake with increasing age so that with a given protein concentration in the diet the older the rat the less protein is ingested each day in relation to body surface. The protein taken in the food has been shown (MacKay and MacKay[3]) to be an important factor in determining the weight of the kidneys. This relationship is expressed for casein as protein intake = 0.0183 renal weight — 2.75. With this formula there are 150 mg. of kidney per 100 sq. cm. body surface with a zero protein intake. Calculated from the latter factor any kidney weight above 150 is then due to the dietary protein. Some of our data have been corrected in this manner and reduced to a zero protein intake basis. The original and corrected data are plotted in Fig. 1.

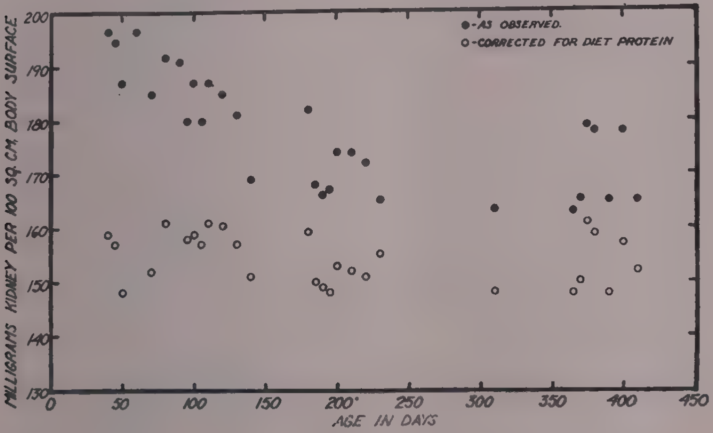

FIG. 1.

When corrected for the effect of the dietary protein the kidney weight (average of both kidneys) in relation to body surface is more nearly the same at all ages. Each point represents an average of from 12-24 male rats.

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[2] MacKay, E. M., and MacKay, L. L., *J. Biol. Chem.*, 1930, **86**, 765.

[3] MacKay, E. M., and MacKay, L. L., *J. Nutri.*, 1931, **3**, 375.

7332 P

Determination of Allantoin in Urine.

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In the course of studies involving allantoin, difficulty was experienced in finding a suitable method for the determination of this substance in urine. The method recently introduced by Larson,[1] while including such favorable features as rapidity and the avoidance of the hydrolysis of allantoin, was not found satisfactory under varying experimental conditions. Since the Folin ammoniacal copper reagent employed in this method is not specific for allantoin, further removal of interfering substances from the urine prior to its addition to the copper reagent seemed desirable. A method involving this modification is the basis of the present report.

Following the precipitation of interfering substances with phosphotungstic acid and lead acetate, the removal of excess of the heavy metals is effected with hydrogen sulphide. The allantoin is then precipitated with mercuric acetate, the precipitate washed and redissolved with hydrochloric acid. The solution is then freed of mercury by means of hydrogen sulphide, and the allantoin in a relatively pure solution is determined colorimetrically with the Folin ammoniacal reagent adopted for this purpose by Larson.

The amount of phosphotungstic acid to be added to the urine is determined by preliminary tests as follows: To a series of 6 tubes add 2 cc. of neutral urine. To the first add one cc. of 10% phosphotungstic acid and to the others increasing amounts, namely 1.5, 2.0, 2.5, 3.0, and 3.5 cc. The tubes are shaken, allowed to stand for 5 minutes, and then filtered. To each of the clear filtrates, a few drops of 10% phosphotungstic acid are added. The least amount of phosphotungstic acid that yields a filtrate remaining clear despite the further addition of the acid determines the amount necessary for precipitation of interfering substances from the urine sample. A large excess of phosphotungstic acid must be avoided since it leads to bulky precipitates, which cause a loss of solution volume. If no one of the filtrates remains clear after the addition of the acid, the urine is too concentrated and must be diluted.

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* Aided by a grant from the Research Board of the University of California, Berkeley.

[1] Larson, H. W., *J. Biol. Chem.*, 1932, **94**, 727.

Ten cc. of neutral diluted urine are transferred to a 15 cc. centrifuge tube and dry phosphotungstic acid is added in an amount determined by the above procedure. · The mixture is thoroughly stirred and allowed to stand for about 15 minutes. It is then centrifuged for 10 minutes. The supernatant fluid is transferred to a 15 cc. graduated centrifuge tube and the volume noted (V_1). The supernatant fluid in every determination is tested with a few crystals of phosphotungstic acid to ensure that all interfering substances precipitable by this reagent have been removed.

One cc. of 50% basic lead acetate is now added to the supernatant fluid in the graduated centrifuge tube. The mixture is thoroughly stirred and tested with litmus paper. If not alkaline, it is made so by the further addition of lead acetate, drop by drop. The volume of lead acetate that has been added is noted (V_L) and the mixture centrifuged. The clear supernatant fluid is transferred to another 15-cc. centrifuge tube and treated with hydrogen sulphide until all the heavy metals have been removed. The precipitate is allowed to settle slightly and with the aid of a glass rod the sulphide clinging to the sides of the tube above the level of the liquid is brushed down. The mixture is centrifuged for about one minute and the supernatant fluid transferred to another 15-cc. graduated centrifuge tube. The solution is aerated for about 15 minutes and the volume noted (V_2). One drop of phenolphthalein is added and the solution titrated with 2 N NaOH until a permanent pink is obtained. Five cc. of a mercury reagent containing 100 gm. mercuric acetate and 300 gm. sodium acetate per liter of solution are now added. The mixture is stirred and allowed to stand for 10 minutes. Following centrifugation, the clear supernatant fluid is discarded. The precipitate is then stirred with alkaline water and the mixture centrifuged. If the urine contains glucose, the precipitate is washed again. The wash water is prepared by adding 5 drops of 2 N NaOH to one liter of water.

Four cc. of water are added to the precipitate, followed by 2 drops of concentrated HCl. The contents of the tube are stirred until the precipitate is dissolved. A faint opalescence may remain at this point. Water is now added to bring the volume to 5.0 cc. (V_3). If allantoin is present in a concentration greater than 0.5 gm. per liter, the volume at this point is made up to 10 cc. The mercury is precipitated with hydrogen sulphide and the tube centrifuged. The clear supernatant fluid is decanted into another tube and aerated for 15 minutes. A 2-cc. sample is then treated with the copper reagent according to Larson's method, and the allantoin content compared colorimetrically with a standard containing 1.0 mg. allantoin.

The allantoin in milligrams in n cc. of diluted urine may be calculated as follows:

$$n \times \frac{S}{U} \times \frac{V_1 + V_L}{V_1} \times \frac{V_s}{V_2} \times \frac{1}{2},$$

where S and U are the readings of the standard and unknown respectively.

7333 P

Effect of Insulin on Excretion of Allantoin by the Normal Dog.

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From the Division of Physiology, University of California Medical School, Berkeley.

The influence of insulin on purine metabolism has been the subject of few investigations. Although Kurti and Gyorgyi[1] found no constant effect of insulin on the endogenous excretion of uric acid in man, they reported that insulin led to an increase in blood uric acid during the ingestion of nucleic acid. Quick[2] has shown that 30 units of insulin along with a high carbohydrate diet had no immediate effect on the uric acid output by man. In the dog, Taubmann[3] reported that insulin led to an increased excretion of allantoin, whereas Ogawa[4] found a decrease following the administration of this hormone.

In the present investigation the dogs were fed a Cowgill diet and insulin was injected after nitrogen and allantoin equilibrium had

TABLE I.
Excretion of Allantoin Nitrogen (Dog. L).

Date	1:35 p.m. to 8:35 a.m.	8:35 a.m. to 1:35 p.m.	Total
Jan. 8	.118	.029	.147
” 9	.112	.026	.138
” 10	.117	.028	.145
” 11*	.119	.062	.181
” 12	.124	.025	.149
” 13	.118	.028	.146

* Insulin injected.

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[1] Kurti, L., and Gyorgyi, G., *Klin. Wochenschr.*, 1927, **6**, 1426.

[2] Quick, A. J., *J. Biol. Chem.*, 1932, **98**, 157.

[3] Taubmann, G., *Arch. f. exp. Pathol. u. Pharmak.*, 1928, **132**, 124.

[4] Ogawa, T., *Folia Endocrinol. Japon.*, 1931, **7**, 7.

been established. Allantoin was determined by the method previously described.[5]

An increased elimination of allantoin following the injection of insulin has been observed in 6 dogs. A typical result is shown in Table I. Dog L was catheterized twice daily, at 8:35 a. m. and at 1:35 p. m., and fed daily at 1:45 p. m. On January 11th, 7 units of insulin were injected at 8:45 a. m. During the 5-hour interval following the injection of the hormone, the excretion of allantoin doubled. Since this effect is produced 19 hours after the ingestion of a purine-poor diet, it would seem that insulin is capable of affecting the endogenous metabolism of purines. In view of the well-known action of insulin hypoglycemia in evoking a secretion of epinephrine, the effect of the latter upon the excretion of allantoin is being investigated.

7334 C

Antihuman Fibrinolytic Streptococci.

J. K. VAN DEVENTER AND T. REICH. (Introduced by W. H. Manwaring.)

From the Laboratory of Bacteriology and Experimental Pathology, Stanford University, California.

Tillett and Garner[1] have recently described a hitherto unknown antihuman fibrinolytic function of hemolytic streptococci. This function is demonstrable with *S. hemolyticus* of human origin, but is not demonstrable with apparently identical environmental or veterinary streptococci. Since the lytic factor is not active against normal rabbit fibrin, many of the results of streptococcus research on rabbits are presumably not applicable to human pathology. We have, therefore, extended the Tillett-Garner tests to include plasma-clots from other animal species. The object of these tests was to find a laboratory animal suitable for a direct study of the topographical distribution[2] and *in vivo* fibrinolytic function of *S. hemolyticus*.

The *S. hemolyticus* used in these tests were 2 human (C 203 and K 96) and 2 veterinary (P 454 and K 158 E) strains kindly furnished by Dr. Lancefield of the Rockefeller Institute. To these we have added a number of local strains, one of special interest being J 1, originally isolated from a clinical case in San Francisco. This strain contains the human-diagnostic carbohydrate fraction recently described by Lancefield.[3]

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[5] Read, L. S., and Chaikoff, I. L., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 818.

[1] Tillett, W. S., and Garner, R. L., *J. Exp. Med.*, 1933, **58**, 485.

[2] Menkin, V., *J. Exp. Med.*, 1933, **57**, 977.

The fibrinolytic tests were made by the Tillett-Garner technic. To 1 cc. 20% oxalated plasma was added 0.25 cc. 0.25% $CaCl_2$-solution, followed by 0.5 cc. 18-24 hour broth culture of the microorganism to be tested. A semi-opaque agar-like clot is usually formed within 10 minutes. In nonlytic or negative tests no softening of this clot is demonstrable after 24 hours incubation at 37°C. In positive tests, beginning (+), well advanced (++), or complete (+++) liquefaction is noted within from 30 minutes to 2 hours.

Tests with plasma-clots from the rabbit, guinea pig, rat, domestic fowl, horse, cow, goat, sheep, dog and cat have all been negative with the 5 streptococcus strains herein reported. Twenty human plasmas and 12 rhesus plasmas have been tested with the same strains. Both human and rhesus fibrins are lysed by the 3 strains of human origin, but are not lysed by the 2 veterinary strains. Rhesus fibrin, however, is invariably dissolved less promptly or less completely than the human control. A typical comparison is recorded in Table I.

TABLE I.
Relative Effects of the Tillett-Garner Streptococcus Lysin on Normal Human and Rhesus Fibrins.

Streptococcus Strain	Lysis of Normal Human Clot					Lysis of Normal Rhesus Clot				
	½ hr.	1 hr.	2 hrs.	6 hrs.	24 hrs.	½ hr.	1 hr.	2 hrs.	6 hrs.	24 hrs.
J 1	+++	+++	+++	+++	+++	±	+++	+++	+++	+++
K 96	+±	+++	+++	+++	+++	0	0	+++	+++	+++
C 203	0	0	+	++	+++	0	0	0	±	++
P 454	0	0	0	0	0	0	0	0	0	0
K 158 E	0	0	0	0	0	0	0	0	0	0

For a direct study of the topographical distribution of *S. hemolyticus* in infected tissues[2] and its *in vivo* fibrinolytic activities, the rhesus monkey is apparently the only readily available laboratory animal which presumably would give results directly applicable to human pathology.*

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[3] Lancefield, R. C., *J. Exp. Med.*, 1933, **57**, 571.

* It should be noted in this connection that Tillett and Garner found that rabbit fibrinogen clotted with human thrombin gives an atypical rabbit fibrin susceptible to the antihuman streptococcus lysin.

7335 P

Development of Anemias in Rats after Exposure to Low Oxygen Tensions.

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From the Department of Zoology, University of Southern California.

We have found that adult rats exposed to low oxygen tension, upon return to normal atmospheric conditions, develop a marked anemia, regardless of whether or not the usual polycythemia occurs.

Sixty-six adult and 11 young rats were exposed to air pressures ranging from 650 to about 360 mm. of mercury for periods of from 2 to 14 days. Red cell counts were made in each case before exposure, immediately after exposure, and then every 48 hours. Two drops of blood were used for the counts and excess bleeding was prevented by temporarily ligating the tail.

The surviving rats responded to the treatment as follows: (1) Twenty-one of the adult rats showed an increase in red cells varying from one to 4 million, following the exposure to low air pressure. Then, within 48 hours, there was a drop amounting to about 45% to 80% of the excess erythrocytes. Later, these rats all developed an anemia lasting in some cases longer than a month, with red cell counts as low as 5 million below the normal. (2) Thirteen adult rats, after similar exposure periods, did not show any apparent increase in the number of circulating red cells. But here again an anemia developed, generally setting in earlier than in the rats of Type 1. (3) Seven young rats, 40 to 60 days of age, with blood counts ranging from 5 to 6½ million, showed increases after the exposure of from 1½ to 4 million red cells. These rats did not develop an anemia with respect to the first blood count. They did show a slight drop after the exposure, followed later by a rise.

Cases of anemias following polycythemias have been reported by many clinicians. "Plethoric anemias" following transfusion of large amounts of blood have been noted.[1-6] In these cases, it has generally been assumed that the cause is an "exhaustion of the bone marrow," a hyperactivity of "blood destroying organs," or an inhibition of the bone marrow by some unknown factors.

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[1] Hess, R., *Deutsch. Arch. klin. Med.*, 1909, **95**, 482.

[2] Itami, S., *Folia haematol.*, 1908, **6**, 425.

[3] Robertson, O. H., *J. Exp. Med.*, 1917, **26**, 221.

[4] Ottenberg, R., and Thalhimer, W., *J. Med. Research*, 1915, **33**, 213.

[5] Krumbhaar, E. B., and Chanutin, A., *J. Exp. Med.*, 1922, **35**, 847.

[6] Kambe, H., and Komiya, E., *Am. J. Physiol.*, 1920, **51**, 1.

It is probable that following the exposure, the tissue oxygen tensions are altered to a higher level than the normal. This increase in the tissue tensions may be partly responsible for the anemia.

7336 P

Comparative Chronic Toxicities of Fluorine Compounds.*

FLOYD DeEDS AND JOHN O. THOMAS.

From the Bureau of Chemistry and Soils, United States Department of Agriculture, at the Department of Pharmacology, Stanford University School of Medicine, San Francisco, California.

The minimal dose capable of producing chronic intoxication in the albino rat has been determined for sodium fluoride, sodium fluosilicate, barium fluosilicate, and sodium aluminum fluoride (natural cryolite). Male rats ranging in weight from 30 to 60 gm. were placed on a basic diet consisting of corn meal 73 gm., linseed oil cake meal 10 gm., alfalfa 2 gm., casein 10 gm., codliver oil 3 gm., bone ash 1½ gm., sodium chloride ½ gm. For each compound studied 6 control rats were placed on this diet, and for each dosage level 6 rats were placed on the same diet to which a weighed amount of the fluorine compound had been added and evenly distributed by means of a mechanical mixer. Analyses showed even distribution of the toxic agent throughout the basic diet. For each compound a series of dosage levels was employed, each dose being one-half the preceding dose.

The criterion of toxic action was bleaching of the upper and lower, especially the lower, incisor teeth. Table I summarizes the data. In the case of each compound, the dosage level indicated means that bleaching was present in all of the rats on that dosage, but absent when one-half of that dose was administered.

When the fluorine content of the molecule is taken into consider-

TABLE I.

Compound	Solubility per 100 parts water	% in diet	Parts of Fluorine per million parts food
Sodium Fluoride	4.000	0.0025	12
Sodium Fluosilicate	0.650	0.0025	15
Barium Fluosilicate	0.026	0.0050	13.7
Sodium Aluminum Fluoride	0.060	0.0050	24.3

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* Food Research Division Contribution No. 221.

ation it is seen that despite a marked variation in water solubility the chronic toxicity of the compounds, expressed as parts of fluorine per million parts of food, is remarkably constant. For all practical purposes, it may be stated that in the compounds studied fluorine is capable of producing chronic intoxication, as indicated by bleaching of the rat incisors, in concentrations as low as 14 to 16 parts per million regardless of the solubility of the compound containing the fluorine. Depending upon the amount of food consumed by the albino rat, this means that from one-half to 1 mg. of fluorine per day per kilo of body weight is capable of producing definite signs of injury to the rat incisors.

Since 2, and possibly one, part of fluorine per million of drinking water is capable of producing mottled enamel in children, and since the average water intake is approximately one liter, it is evident that an intake of one to 2 mg. of fluorine per diem is toxic to a child. Accordingly, human subjects appear to be more susceptible to fluorine poisoning than do rats, and the data obtained on rats become all the more significant from the public health standpoint.

7337 C

Acute Toxicity of Trypan Blue, Gentian Violet and Brilliant Green.*

HAMILTON H. ANDERSON, GEORGE A. EMERSON, AND B. H. FISHER.

From the Pharmacological Laboratory, University of California Medical School, San Francisco.

Difficulties encountered in administering sufficient amounts of the usual chaulmoogra oil derivatives uniformly to effect a cure in leprous subjects have led to the attempted application of other forms of therapy. Thus, recent clinical developments[1, 2] again have aroused interest in the efficacy of certain non-chaulmoogryl drugs, synthetic dyes in particular, against leprosy. In the course of examining the possible antileprotic activity of certain such compounds by a standardized technique,[3] determination of their toxicity in the test animals was a necessary preliminary. As trypan blue, gentian violet, and brilliant green have been used in an empirical manner in the clinic before a critical pharmacological evaluation of their worth has been made, it is advisable to present the results of this toxicity study on experimental animals, before toxic doses may unavoidably be given to human leprous subjects. The intravenous dosage of these dyes, as reputedly used by Ryrie[1] and Ryles,[2] is within the possible lethal range as estimated from experimental animals. While these workers do not give the sources of the dyes used by them, nor their standards of purity, so that it cannot be judged whether or not their dyes are comparable in purity to those used by us, it is significant that Ryrie[1] admits the occurrence of dangerously severe acute toxic effects in his patients.

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*Part of a cooperative study of the chemotherapy of leprosy conducted by the Pacific Institute of Tropical Medicine within the Hooper Foundation for Medical Research, and the Pharmacological Laboratory of the University of California Medical School, San Francisco, and supported in part by the Christine Breon Fund for Medical Research and Eli Lilly and Co., Indianapolis.

[1] Ryrie, G. A., *Trans. Roy. Soc. Trop. Med. and Hyg.*, 1933, **27**, 85.

[2] Ryles, C. S., *Leprosy Review*, 1933, **4**, 90.

Previously reported toxicity data on these 3 dyes are so inadequate and variant that a recent encyclopedic work on chemotherapy[4] omits all reference to their toxicity. Discrepancies in some cases may be explained by variations in purity and by the use of old solutions. Photodynamic action is not a significant factor in the toxicity of these dyes as far as we could determine. We will later make a more extended report surveying the therapeutic effectiveness as well as the chronic and acute toxicity of these agents. Such a study is indicated in view of enthusiastic endorsement of dye therapy in leprosy on grounds that it is as good as treatment with hydnocarpus oil derivatives, *safe*, cheap and not unduly painful.

In Table I may be found our toxicity observations. The variation in toxicity with mode of administration emphasizes the difficulty of absorption of compounds of such high molecular weight. Particularly marked is the absence of toxic effects on oral administration due to negligible assimilation from the gut. On the other hand, when given by other routes some excretion of the dyes into the gut has been noted. Examination of the data in Table I discloses the wide toxic range of all these agents, which suggests caution in human therapy. Also, considerable species variation is encountered, with the guinea pig being generally more susceptible. Special interest may be attached to the variation in intravenous toxicities of trypan blue in different species, since it is by this route that it is recommended for human therapy.

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[3] Anderson, H. H., Emerson, G. A., and Leake, C. D., *Internat. J. Lep.*, in press.

[4] Fischl, V., and Schlossberger, H., *Handbook of Chemotherapy*, Vol. 1, Baltimore, 1933.

TABLE I.

Acute Toxicity of Trypan Blue, Crystal Violet, and Brilliant Green in Various Species of Animals by Various Routes of Administration.

Animal	% Aqueous Solution	Method of Administration	Dosage (mg./Kg.) and Mortality Ratio	
Trypan Blue: National Aniline Chemical Co., 90% C. P.				
Mouse	1	Intraperitoneal	350 400	2/5 5/5
	1	Intravenous	100 200	1/5 3/5
Rat	5-10	Oral	Not absorbed (1 gm./Kg. tolerated)	
	2	Subcutaneous	300 400	2/5 4/5
	2	Intraperitoneal	300 350	2/5 5/5
	2	Intravenous	200 300	0/5 5/5
Rabbit	5	Intraperitoneal	300 400	0/3 3/3
	5	Intravenous	100 150	1/5 3/5
Guinea Pig	2	Subcutaneous	200 300	0/5 2/3
	2	Intraperitoneal	200 250	1/5 3/6
Crystal Violet: Coleman and Bell, 80% C. P.				
Mouse	0.1	Intraperitoneal	10 20	1/5 5/5
	0.1	Intravenous	10 20	0/3 2/3
Rat	1	Oral	250 —	0/3 —
	0.5	Intraperitoneal	10 15	1/5 3/5
Guinea Pig	1	Intraperitoneal	5 10	0/3 3/3
Brilliant Green: Coleman and Bell, Medicinal Grade				
Mouse	0.1	Intraperitoneal	3 5	0/3 4/5
	0.1	Intravenous	1 3	0/3 4/4
Rat	1	Intraperitoneal	5 8	0/5 4/5
Guinea Pig	1	”	1 3	0/5 4/5

Pathological examination of animals dying of fatal doses has not elucidated the mechanism of toxic action. After such doses the dye is regularly distributed throughout the tissues, with some segregation in the reticulo-endothelial system noted only with trypan blue. Brilliant green and gentian violet appear to damage the liver relatively more than does trypan blue, which has been found in considerable quantity in the kidneys especially intracellularly in the descending loop. In all animals dying of toxic doses, marked congestion of the lungs was noted. Lengthening of the coagulation time of the blood of treated animals has been reported for trypan blue,[5] while on prolonged administration of this agent to rabbits we have noted grossly a shortening of coagulation time after 2 acute toxic doses are administered in divided amounts over a period of 3 weeks. Certain observations lead us to suspect central nervous system depression in trypan blue treated animals; the brain and spinal fluid are reported to be impermeable to gentian violet, which is rapidly fatal in small amounts intracisternally.[6]

Experimental therapy on leprous rats has shown gentian violet and brilliant green to be too toxic for continued administration, while trypan blue, and other dyes which seemed preferable to us[7] because of their high leprocidal activity, are better tolerated.

7338 C

An Improved Method for In Vitro Cultivation of B. Leprae.*

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From the Department of Pathology and Bacteriology, Tulane University.

Many attempts have been made by various investigators to culture Hansen's bacillus of human leprosy. Undoubtedly, in a number of these attempts the investigators have mistaken the increase in number of acid-fast rods, which occurs in removed autolysing pieces of leprous tissue independent of any specially supplied cultural conditions, for multiplication *in vitro* upon artificially prepared nutrient medium. The failure to appreciate the facts that *B. leprae* continues to multiply and that growth ceases when the leprous tissue autolysate is no longer available, explain the increasing difficulty to obtain macroscopic growth of the specific microorganism in transplants through successive generations, and accounts for the absence of growth in subculture in or upon a medium that does not contain the split protein products (amino-acids).

One of us (Duval[1]) reported that *B. leprae* seemed unable to

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[5] Hugget, A. St. G., and Rowe, F. M., *J. Physiol.*, 1933, **80**, 82.

[6] Kolmer, J. A., *Principles and Practice of Chemotherapy*, Philadelphia, 1926, p. 94.

[7] Emerson, G. A., and Salle, A. J., PROC. SOC. EXP. BIOL. AND MED., 1934, **31**, 428.

* Aided by a grant from David Trautman Schwartz Research Fund.

[1] Duval, C. W., *J. Exp. Med.*, 1910, **12**, 649.

utilize whole protein *in vivo*. This was substantiated by repeated observations that the Hansen bacillus would grow in transplanted leprous tissue after autolysis had commenced. The initial culture away from the influence of the host-tissue was possible if the artificial culture medium contained certain split-products of protein. In our recent studies upon the cultivation of *B. leprae,* we have modified Duval's[2] earlier method. An improved procedure as described below is recommended.

The uncontaminated subcutaneous leprous nodule, rich in acid-fast bacilli, is the material of choice. Contaminated tissue is unsatisfactory, because one is likely to injure or destroy the viability of the Hansen bacillus when killing the contaminants. The use of sodium hydroxide, or the like, therefore, is not recommended as a germicide. The skin over the selected leprous nodule is cleansed with soap and water and then rinsed with alcohol. Mercurochrome, iodine or other antiseptics should not be employed. The overlying skin is next incised and the exposed nodule is dissected free and removed under strict asepsis.

The removed nodule is cut into small pieces (circa 4 mm. diameter) with sterile scissors and placed in a 1% trypsin solution previously made sterile by passage through a Berkefeld V or Seitz filter. The trypsin immersed leprous tissue bits are incubated at 37°C. for 36 to 48 hours. This period of incubation is sufficient to digest them so that their consistence is of soft butter. When the softened bits are removed and placed on the surface of slanted agar, they are readily spread with an ordinary platinum loop.

Smear-preparations are made from the trypsinized leprous material and stained by Ziehl-Neelsen's method to observe the morphology and to determine the approximate number of acid-fast rods per microscopic field. These stained preparations afford an index to the subsequent multiplication of *B. leprae*. At this time, control cultures are also made to determine possible bacterial contamination. However, when the leprous tissue contains ordinary extraneous bacteria, they evidence themselves by clouding the trypsin fluid used as a tissue digestant. It should be mentioned here that 1% trypsin solution does not destroy or inhibit the growth of common bacteria, nor has it any detrimental effect upon *B. leprae*.

The uncontaminated trypsinized leprous pieces of tissue which now have the consistence of soft butter are carefully transferred and smeared over the surface of slanted amino-acid agar by means of a sterile platinum loop. The inoculated culture tubes are plugged with paraffined cotton to prevent undue evaporation during the long period of incubation at 37°C. To permit the entrance of fresh atmosphere into the culture tubes, the paraffined cotton plugs at intervals of 3 to 5 days are removed from the tubes and replaced before the paraffin cools.

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[2] **Duval, C. W., *J. Exp. Med.*, 1911, 13, 365.**

The split protein products are prepared separately in the form of clear neutral filtrates by dissolving the powders or crystals (C.P.) in sterile distilled water to the point of saturation. These filtrates are sterilized by passage through Berkefeld V or Seitz filters.

Agar is used only as a menstrum for the special nutrients. It is prepared in 3% concentration to allow for dilution with the various filtrates. Ordinary agar-powder is melted in Ringer's solution, cleared with whole egg, titered to pH 7, filtered, tubed in the desired quantity and sterilized in the autoclave.

The placenta autolysate is obtained by mixing 500 gm. of ground human placenta in 500 cc. of Ringer's solution. The infusion is allowed to stand for 10 days at refrigerator temperature. It is first filtered through cotton to remove the solid particles, then passed through a Seitz filter. The sterile filtrate constitutes the placenta autolysate.

The leucocytic extract is secured by infusing for 10 days at refrigerator temperature 500 gm. of ground human pneumonic lung (grey stage of hepatization preferred) in 500 cc. of Ringer's solution. It is then filtered through Berkefeld V or Seitz filter and the filtrate is designated as the leucocytic extract.

Banana extract is prepared by emulsifying 500 gm. of peeled over-ripe bananas in 500 cc. Ringer's solution. The emulsion is first filtered through cotton and then through the Berkefeld or Seitz filter to obtain the sterile amber color filtrate which is designated as the banana infusion.

The procedure and proportions of ingredients employed in the preparation of the special split protein medium are as follows: to individual tubes containing 5 cc. of melted 3% sterile neutral agar,

	cc.
Tryptophane, 2.0% aq. sterile filtrate (Tryptophane solubility, 20 mg. per cc. dist. H_2O).	2
Cystine, 0.1% aq. sterile filtrate (Cystine solubility, 0.1 mg. per cc. dist. H_2O).	2
Tyrosine, 0.5% aq. sterile filtrate (Tyrosine solubility, 0.5 mg. per cc. dist. H_2O).	2
Leucine, 2.0% aq. sterile filtrate (Leucine solubility, 20 mg. per cc. dist. H_2O).	2
Placenta autolysate or leucocytic extract, sterile filtrate	1
Banana infusion, sterile filtrate	0.5
Glycerine, sterile	0.5

the various quantities of the special ingredients are added. Each ingredient is added separately under strict asepsis to the melted agar which is kept liquid in a hot water bath between the filling procedures. Upon adding the last ingredient, the tube is slanted to allow the content to solidify in this position.

Summary. The trypsinization of the leprous tissue bits, before attempting the cultivation of the contained *B. leprae,* affords at once the split-protein products which we consider the necessary nutrient factor. The trypsin apparently exerts no harmful effect upon the Hansen bacillus as evidenced by its unmistakable growth in 4 to 6 weeks. This initial *in vitro* multiplication occurs in the trypsinized leprous tissue without the aid of other nutrients and under ordinary atmospheric conditions at 37°C. Growth continues in subculture on ordinary agar medium as long as the trypsinized leprous material is transferred. The crucial point in the initial cultivation of *B. leprae* away from the influence of the host tissue is experienced at this juncture. After the complete utilization of the nutrient material of the trypsinized host tissue, the acid-fast bacillus of Hansen will only multiply in a medium that is enriched with sufficient quantities of protein cleavage products.

7339

Repeated Determinations of Pulse Wave Velocity on Normal Individuals.*

ROY H. TURNER AND W. A. SODEMAN.

From the Department of Medicine and the Hutchinson Memorial Clinic of Tulane University of Louisiana, New Orleans.

The existing literature concerning pulse wave velocity deals with the effect of disease, age, sex and physiologic and pharmacologic stimuli on pulse wave velocity in groups of individuals. Spontaneous fluctuations in the same individual from time to time have received slight consideration.[1, 2, 3, 4]

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* Aided by grants from the David Trautman Schwartz Research Fund and grant No. 163 of the Committee on Scientific Research of the American Medical Association.

[1] Beyerholm, O., *Acta Med. Scand.*, 1927, **67**, 203.

[2] Bazett, H. C., and Dreyer, N. B., *Am. J. Physiol.*, 1922, **63**, 94.

[3] Schafer, E. A., *Textbook of Physiology*, Vol. II, Edinburgh, 103.

[4] Keyt, A. T., *N. Y. Med. J.*, 1878, **28**, 30.

TABLE I.

B.P.	Mean P.W.V. Meters/sec.	High - Low		
Subject 1, male, aged 39.				
112/74	6.84	6.16 - 7.28	C-R I*	
110/80	13.3	11.48 - 17.2	C-R I	
not rec.	7.38	7.30 - 7.91	C-R I*	
” ”	6.17	5.82 - 6.53	C-R I*	
115/80	8.17	7.73 - 8.89	C-R I	
115/80	8.41	8.1 - 8.95	C-R I	
118/90	6.64	6.17 - 6.93	C-R I	standing
116/84	6.33	6.18 - 6.70	C-R I	lying
108/76	8.47	7.75 - 8.96	C-R R	
106/84	14.4	10.9 - 17.0	C-L I	
Subject 2, male, aged 27				
117/88	10.9	9.8 - 11.5	C-R I	
118/84	7.22	7.13 - 7.62	C-R I*	
not rec.	9.05	8.45 - 9.6	C-L I	
117/88	11.2	10.5 - 12.1	C-L I	
118/88	7.62	6.82 - 7.83	C-L I*	
not rec.	8.88	7.85 - 9.80	C-R R	
110/85	6.45	5.8 - 7.24	C-R R*	
Subject 3, female, aged 27				
102/80	5.75	5.20 - 6.64	C-R I	
100/62	6.86	6.67 - 7.37	C-R I*	
102/78	8.03	7.30 - 9.14	C-L I	
100/62	7.00	6.67 - 7.37	C-L I	
110/80	5.7	5.3 - 6.23	C-R R	
90/70	6.63	6.23 - 7.07	C-R R*	
110/80	8.41	7.58 - 9.63	C-L R	
97/75	5.0	4.77 - 5.25	C-L R*	
95/70	5.55	5.25 - 6.19	C-L R*	
Subject 4, female, aged 22				
90/65	5.78	5.30 - 6.32	C-R I*	
90/65	7.85	7.35 - 8.80	C-L I*	
95/60	7.03	6.5 - 7.43	C-R R	
95/60	5.72	5.47 - 6.12	C-L R	
100/68	5.82	5.55 - 6.25	C-L R*	
Subject 5, female, aged 45				
115/80	7.87	7.5 - 8.06	C-R I*	
115/80	6.80	6.45 - 7.18	C-L I*	
122/86	7.02	6.73 - 7.84	C-R R	
122/86	8.30	7.23 - 9.40	C-L R	
110/83	6.25	5.94 - 6.33	C-L R*	
Subject 6, female, aged 24				
100/74	8.70	7.55 - 9.13	C-R I	
102/72	7.55	6.45 - 8.65	C-L I	
112/80	6.42	6.27 - 6.57	C-L I	standing
106/75	6.93	6.00 - 7.6	C-L I	lying
98/60	11.5	10.8 - 13.5	C-R R	
98/60	9.15	8.15 - 9.64	C-L R	
105/68	5.45	5.40 - 5.68	C-L R*	

Mean P.W.V. is the average of 6 to 18 consecutive complexes in all phases of respiration.

High-low = highest and lowest P.W.V. in each record.

C-R I = left carotid-right index fingertip; C-L R = left carotid-left radial; C-R R = left carotid-right radial; C-L I = left carotid-left index fingertip.

* = determinations in which conditions approached those used in determination of basal metabolism and cardiac output.

We have measured repeatedly during a period of 6 months the pulse wave velocity in 6 normal individuals of 22 to 45 years of age. A simplified modification of a technic previously described[5] was used, which employs carbon grain microphones and 2 Einthoven string galvanometers. Carotid and either radial or index fingertip sphygmograms, together with respiratory movements, were recorded simultaneously and measurements made from reference points on the ascending limbs one-fifth of the vertical distance from beginning of upstroke to the peak. Brachial blood pressure was taken immediately following the taking of the record. With a camera speed of 10 cm. per second measurements were made with accuracy to 0.002 second. Subjects were examined at approximately the same time of day, in the sitting position (unless indicated otherwise) with the arms resting horizontally on supports. Preliminary rest periods varied from 5 to 45 minutes. Room temperatures were fairly constant. Noises and other demands upon the subjects' attention were kept at a minimum.

Careful scrutiny of Table I shows that for the group as a whole there is no apparent correlation between pulse wave velocity and systolic and diastolic pressure. When pulse wave velocity for each subject was plotted against systolic and diastolic blood pressures the following relationships were noted:

Subject	Systolic	Diastolic	Subject	Systolic	Diastolic
No. 1	—	±	No. 4	±	±
No. 2	±	±	No. 5	+	±
No. 3	±	±	No. 6	—	—

+ = P.W.V. tends to increase with B.P.
— = P.W.V. tends to diminish with B.P.
± = No apparent correlation.

Most workers[2, 3, 6, 7] seem to agree that in normal arteries blood pressure is the principal factor which influences pulse wave velocity and that as blood pressure varies pulse wave velocity also varies and in the same direction. Some observers are not in full accord.[8, 9, 10]

We have noted in individuals with apparently normal arteries and with normal blood pressure pulse wave velocity which was extremely high even when the blood pressure was lower than usual for that individual. A related observation has been made before in this laboratory[11] with a less satisfactory method when pulse wave velocity in patients with hypertension actually increased following a drop in pressure produced by nitrites.

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[5] Turner, R. H., *Johns Hop. Hosp. Bull.*, 1928, **43**, 1.

[6] Hickson, S. K., McSwiney, B. A., *J. Physiol.*, 1924, **59**, 217.

[7] Laubry, C., Mougeot, A., and Giroux, R., *Arch. d. med. du Coeur*, 1922, **14**, 49, 97.

[8] Fulton, J. S., and McSwiney, B. A., *J. Physiol.*, 1930, **69**, 387.

[9] Dawson, *Am. J. Physiol.*, 1917, **62**, 613.

[10] Hafkesbring, R., and Ashman, R., *Am. J. Physiol.*, 1932, **100**, 89.

The observations here reported and others to be published later indicate to us that changes in arterial tone largely independent of the blood pressure may greatly influence pulse wave velocity in the brachial and radial arteries. Such a concept gives to the artery not a passive rôle, merely distended by its internal pressure, but ascribes to it an active dynamic rôle more in keeping with its known muscular equipment. Final interpretation of data such as here presented is impossible without knowledge of the changes in arterial calibre which is an important variable influencing pulse wave velocity.

It was noted also that as the conditions of the experiment approach more closely the basal conditions laid down for the determination of basal metabolism and cardiac output that pulse wave velocity became lower and less variable.

7340 P

Relation of Adrenals to Diabetes.*

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Frouin[1] found that pancreatectomy produced less glycosuria than usual, in dogs from which a considerable amount of adrenal tissue had been removed. Later work[2] has indicated an interrelationship between the adrenal and the pancreas. The adrenal cortex is necessary for the development of severe diabetes.[3] The medulla, however, may be significant in preventing insulin shock.[4]

If the cortex is necessary for the full development of diabetes, is its cortin-producing or some other function responsible?

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[11] Turner, R. H., and Herrmann, G. R., *J. Clin. Invest.*, 1927, **4**, 430.

* Aided by a grant from the Carnegie Corporation of New York to the Carnegie Institution of Washington.

[1] Frouin, A., *Compt. rend. Soc. de Biol.*, 1908, **64**, 216.

[2] Riddle, O., Honeywell, H. E., and Fisher, W. S., *Am. J. Physiol.*, 1924, **68**, 461.

[3] Turcatti, E. S., *Rev. Méd., Rosario*, 1929, **19**, 269.

[4] Boggild, D. H., *Acta Med. Scand.*, 1933, **79**, 458.

We have studied 9 cats in an attempt to throw light upon this problem. In 3 animals the right adrenal and part of the pancreas, then several days later the left adrenal and the rest of the pancreas, were removed. Two died in less than 24 hours, with blood sugars of 49 and 61 mg. per 100 cc. respectively. The third survived 6 days. Injected with cortin it showed a small increase in blood sugar from 98 to 156 mg., and slight glycosuria.

The remaining adrenal of 2 pancreatectomized, unilaterally adrenalectomized cats was denervated after establishment of diabetes. Eight days after denervation one animal showed reduction in diabetes; the other showed no reduction for 3 weeks and then only after cautery of the medulla and much of the adrenal cortex.

Four cats were kept alive by cortin injections after removal of the second adrenal, the pancreas having been removed in a 2-stage operation at the time of the adrenalectomies.

One cat showed some reduction in the glycosuria after the second adrenal was removed. Death occurred on the eighth day, perhaps from insulin, which was injected for the first time on that day.

The second cat refused to eat following the removal of the second adrenal. The high blood sugar (279 mg. per 100 cc.) and the glycosuria (maximum 290 mg. of glucose per hour) did not fall during the time of observation (4 days). He was killed in another experiment.

The third cat, kept alive with cortin for 13 days, showed some reduction in diabetes after the second adrenal was removed. The amount of sugar in the blood and urine was then determined at least in part by the amount of cortin, as well as the food eaten. With cortin insufficiency appetite fell and glycosuria dropped to little or none. Resumption of cortin treatment brought recurrence of diabetes although not to the former level.

The fourth animal was studied for 2 months, 38 days after total adrenalectomy. For 3 weeks or more during this latter period there was no reduction in diabetes but after this time there was marked reduction which large amounts of cortin failed to restore.

Our results indicate that cortin is essential for the maintenance of diabetes.

7341 P

Cell Lipids and Function in Muscle.

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A definite correlation between phospholipid content and function in muscle was shown by the results of a comparison of the lipid content of various muscles of wild vs. laboratory rabbit, and hen vs. pigeon. In every case the trained muscles, such as the muscles used in running and jumping by the wild rabbit, had a much higher content of phospholipid than the same muscles in the untrained laboratory animal. The muscles used in flying by the pigeon had a much higher phospholipid content than the same muscles in the domestic hen. The cholesterol content of the muscles in the 2 groups of animals was not definitely different so that the ratio, phospholipid/cholesterol, was always higher in the trained muscle than in the untrained muscle.

Tables I and II will illustrate the differences found.

TABLE I.
Phospholipid Content of Muscles.
(Expressed as phospholipid fatty acids percentage in terms of dry weight.)

	Laboratory Rabbit	Wild Rabbit
Heart	4.0	5.3
Jaw	4.5	2.6
Neck	2.1	3.0
Diaphragm	2.4	3.6
Front Leg	2.0	2.8
Abd. Wall	2.7	3.4
Thigh	1.2	3.4
Back	0.9	2.6

TABLE II.
Pl. F. A. % Dry Weight in Muscles of Birds.

	Hen	Pigeon
Heart	4.0	6.0
Neck	1.6	2.7
Thigh	3.0	2.0
Front Limb	1.2	3.2
Abd. Wall	2.3	3.3
Pector. Maj.	1.5	4.0
Pector. Min.	1.1	2.7

7342

Observations on Coronary Occlusion III: Chemical and Histologic Changes.*

D. M. GRAYZEL, R. TENNANT, S. W. STRINGER AND F. A. SUTHERLAND.
(Introduced by R. Hussey.)

From the Departments of Pathology and Surgery, Yale University School of Medicine.

A series of experiments was made to study the chemical changes with reference to lactic acid and glycogen in the myocardium following ligation of the anterior descending branch of the left coronary artery of dogs and to correlate these with the anatomic and electrocardiographic changes observed.

Sixteen dogs were used. Essentially similar operative techniques were employed as in the experiments previously reported.[1, 2] The tissues for chemical studies were excised from the heart *in situ* in the living animal under amytal anesthesia and immediately plunged into liquid air. Lactic acid was determined by the method of Friedemann and Graeser[3] and the glycogen by the modified Pflüger procedure as outlined by Yannet and Darrow.[4]

In the first group of experiments 10 dogs were used. After ligation of the coronary vessels the animals were sacrificed at intervals varying from ½ hour to 24 hours. The lactic acid content of the muscle tissue from the zone supplied by the ligated vessels was increased 100-200% over that of the control zone (right ventricle) while the glycogen content of similar zones was decreased below that of the control zone. These changes were noted in every instance irrespective of the duration of the period of ligation. Control animals subjected to the same operative procedure except that the sutures were passed about the vessels and then removed, showed no significant variation in the content of lactic acid or glycogen from the two sides of the heart.

In the second group of experiments the coronary vessels were ligated for varying periods up to 8 hours. At the end of these in-

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* This investigation is supported by funds from the Josiah Macy, Jr., Foundation.

[1] Sutherland, F. A., Dial, D. L., and Harris, B. R., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1430.

[2] Harris, B. R., Sutherland, F. A., Ramsey, E. M., and Gaiser, D. W., PROC. SOC. EXP. BIOL. AND MED., 1933, **31**, 222.

[3] Friedemann, T. E., and Graeser, J. B., *J. Biol. Chem.*, 1933, **100**, 291.

[4] Yannet, H., and Darrow, D. C., *J. Clin. Invest.*, 1933, **12**, 779.

tervals the ligatures were removed and the animals were sacrificed 2 hours afterward. Six dogs were used in this series. In all instances the lactic acid content of the heart muscle from the zone supplied by the previously ligated vessels was decreased below that of the muscle from the control zone. The glycogen content of the affected zones, as in the first group, was also decreased below that of the control zone.

In the first group of experiments the anatomic changes were similar to those previously reported.[1] No demonstrable characteristic alteration was observed in the myocardium of animals surviving for 10 hours or less. In contrast to these findings the second group showed striking gross and microscopic changes in every case except in the instance when the vessels were tied for only ½ hour. In all the other hearts a sharply delimited zone, extending from the apex for a distance of 3 cm. up the anterior surface of the left ventricle was seen. The myocardium here was soft, dark red and sharply delineated from the adjoining red-brown myocardium. The dark red discoloration extended through the entire thickness of the wall with the most intense involvement in the inner half. The anterior one-third of the interventricular septum was also involved by this process. The histologic changes were the same in all. They consisted of edema, extensive hemorrhage and a slight polymorphonuclear cellular exudate distributed diffusely throughout the interstitial tissue of the involved zone. In the region where the exudate and hemorrhage was most marked the myocardial fibers stained intensely pink and their striations were lost. Fat droplets were not present in any of the muscle fibers.

The electrocardiographic changes in this series are similar to those previously reported,[2] except that following the removal of the ligatures the development of ectopic ventricular premature beats and tachycardia was observed.

7343 C

Vitamin C and the Adrenal Gland in the Dog.*

HARRY M. VARS AND J. J. PFIFFNER.

From the Biological Laboratory, Princeton University.

Harris and collaborators[1, 2] have shown that dogs do not require vitamin C in their diets for adequate nutrition. Others have kept dogs upon synthetic rations, presumably devoid of this vitamin, for periods of 1-4 years without symptoms of scurvy. Tissues from such animals, notably the adrenals and liver, show by chemical and biological tests the presence of large amounts of ascorbic acid. Similar tissues from guinea pigs kept upon scorbutic diets reveal a marked diminution in the content of ascorbic acid as scurvy develops.

The early observation[3] that the cortex of the adrenal gland contained large amounts of "hexuronic acid", later shown to be vitamin C[4, 5, 6] has led to speculation upon the relationship of this vitamin to the functions of the adrenal cortex. The belief that the medulla[7] did not contain "hexuronic acid" supported the idea of a functional significance for this uneven distribution. It has since been demonstrated that other animal tissues contain appreciable amounts of ascorbic acid, *e. g.,* liver,[8] corpora lutea,[9, 10] pituitary[11] thymus.[12] Huszák[13] has shown that the adrenal medulla contains as much vitamin C as does the cortex. Such a wide distribution is hardly indicative of a specific relationship between vitamin C and the functions of the adrenal cortex. In those species that do not develop scurvy one may reasonably consider any tissue containing much vitamin C as having a possible rôle in protecting the animal from this nutritional disorder.

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* This investigation was supported by the Josiah Macy, Jr., Foundation.

[1] Harris, L. J., quoted by Innes, *Ann. Rep. Dir. Inst. Path.,* Cambridge, 1931, pp. 143-50.

[2] Harris, L. J., and Ray, S. N., *Biochem. J.,* 1933, **27**, 580.

[3] Szent-Györgi, A., *Biochem. J.,* 1928, **22**, 1387.

[4] King, C. G., and Waugh, W. A., *Science,* 1932, **75**, 357.

[5] Svirbely, J. L., and Szent-Györgyi, A., *Nature,* 1932, **129**, 576.

[6] Waugh, W. A., and King, C. G., *Science,* 1932, **76**, 630.

[7] Szent-Györgyi, A., *Science,* 1930, **72**, 125.

[8] Svirbely, L. L., *Biochem. J.,* 1933, **27**, 960.

[9] Huszák, St., *Z. Physiol. Chem.,* 1933, **219**, 275.

[10] Bessey, O. A., and King, C. G., *J. Biol. Chem.,* 1933, **103**, 687.

[11] Gough, J., and Zilva, S. S., *Biochem. J.,* 1933, **27**, 1277.

[12] Euler, H., and Klussman, E., *Z. Physiol. Chem.,* 1933, **219**, 215.

[13] Huszák, St., *Z. Physiol. Chem.,* 1933, **222**, 229.

The question arises, is the adrenal gland in the dog concerned specifically with the synthesis and metabolic functioning of vitamin C and the consequent protection of the animal from scurvy?

Six adult male dogs have been adrenalectomized and kept alive with cortical hormone for periods of 15-33 months without any of the commonly recognized symptoms of scurvy (these animals are being used for assay of hormone preparations). During this time their diets have been almost exclusively proprietary dog foods. Only 2 animals have been varied from these diets at any time; one received a lean-meat liver diet for one month 28 months ago, while the other received beef heart for a similar period 17 months ago.

The foods have been tested for vitamin C. Bal-Ra† (Diet I) and Ken-L-Ration‡ (Diet II) were supplemented with yeast,§ salt mixture[14] and cod liver oil to make the rations similar to those fed the dogs. The assays upon guinea pigs indicate clearly that the foods are devoid of this dietary adjuvant.

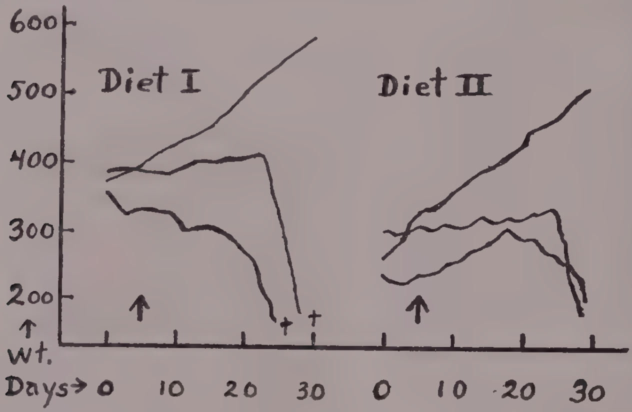

CHART 1.
Typical weight curves of guinea pigs on scorbutic diets of Bal-Ra (Diet 1) and Ken-L-Ration (Diet II).
At ↑ cabbage withdrawn from diets of experimental pigs, control I continuing on 10 gm. cabbage daily, control II transferred to 2 cc. orange juice daily.

Chart 1 gives the details of typical weight curves (selected from data on 18 animals). The 2 experimental animals on diet I died in 23 and 27 days respectively, exhibiting all of the classical symptoms of scurvy. Those on diet II showed the same early symptoms, and when death was imminent (28th day) both were killed and autopsied. The controls were autopsied at the same time. No evidences of scorbutic lesions were observed in the controls while the experimental animals showed the usual severe abnormalities. Determination of the vitamin C content of the adrenal glands, by the technique of Birch, Harris and Ray[15] gave for the controls 0.38, and the experimentals 0.09 mg. per gm. tissue. These figures compare favorably with data reported by others. The growth curves of the control pigs indicate that the failure of the experimental animals was not due to toxic effects of the diet, *e. g.*, animal protein content.

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† Valentine Meat Juice Co., Richmond.

‡ Chappel Brothers, Rockford.

§ Northwestern Yeast Co., Chicago.

[14] Cowgill, G. R., *J. Biol. Chem.*, 1923, **56**, 735.

The method of preparation of the adrenal cortical hormone precludes the presence of more than insignificant traces of ascorbic acid. Three guinea pigs, after the tenth day on a scorbutic diet, received 30 dog units of cortical hormone daily. They were killed on the 15th day of injection. No differences were observed in the weight curves or in the ascorbic acid content of the adrenals and livers of experimental and control animals. All showed marked scorbutic lesions. In this instance the injection of cortical hormone did not retard the development of scurvy. Hartman[16] considers that the cortical hormone delays the onset of scurvy.

The feeding of large amounts of vitamin C to the adrenalectomized dog does not cause any change in the hormone requirement. An assay was repeated on 2 dogs using the same extract. In the latter assay they received daily 5 mg. ascorbic acid per kilo (decitrated concentrated lemon juice) during the whole course of the experiment, *i. e.*, 30 days. The failing dose was the same in both cases. No change was noted in any of the symptoms accompanying insufficiency that would indicate a beneficial effect of the vitamin administered.

Conclusion. No evidence has been obtained that the adrenal gland, as an organ in the dog, is concerned with either the synthesis or the metabolism of vitamin C. If the adrenals do play any rôle in the metabolism of this compound the action is conferred by the adrenal cortical hormone.

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[15] Birch, T. W., Harris, L. J., and Ray, S. N., *Biochem. J.*, 1933, **27**, 590.

[16] Lockwood, J. E., and Hartman, F. A., *Endocrinology*, 1933, **17**, 501.

7344

A Gonad-Stimulating Extract from Alfalfa Meal.*

MAURICE H. FRIEDMAN AND GERTRUDE S. FRIEDMAN. (Introduced by H. C. Bazett.)

From the Department of Physiology, University of Pennsylvania Medical School.

For reasons which can better be stated in a more complete report, it was thought highly probable that the pituitary hormone was not elaborated in the animal body from simple chemical substances, but on the contrary, was built up from complex precursors, or, taken in from the environment in a form not far removed from the hormone itself. If such hypothesis were correct, one should be able to isolate from foods these complex substances, or precursors, which when given to an animal in sufficient amounts, would prove to be gonad-stimulating. Of the foodstuffs considered, green plants seemed to be the most likely source of such material. Consequently, alfalfa was investigated from this standpoint and found to yield an extract capable of affecting the rabbit ovary in a fashion similar to that of the material found in pregnancy urine; *i. e.,* the production of corpora lutea and corpora hemorrhagica in unmated rabbits.

The extract is prepared by mixing 1 kilo of alfalfa meal with 10 liters of water and sufficient 3N alkali to bring the reaction to pH 9-10. After 4 hours the mixture is filtered by suction through a mat of moistened alfalfa meal, and the filtrate then subjected to the Katzman-Doisy benzoic acid method for the extraction of the active material from pregnancy urine.[1] The extracts prepared so far are highly toxic so that they can not be injected intravenously, and therefore cannot be satisfactorily assayed by the rabbit method. The material must be injected either intraperitoneally or subcutaneously, and in order to obtain a positive response it has been necessary to inject the equivalent of 300 to 500 gm. of dry alfalfa in 2 doses not more than 12 hours apart. From previous experience with injections of pregnancy urine by the intraperitoneal route, it is estimated that the yield from 1 kilo of dry material is probably more than 30 rat units and probably less than 100 rat units. The negative results obtained with the injections of inactive extracts, prepared in an identical manner, except for 24 hours (or more) initial exposure to the dilute alkali instead of 4 hours, together with the intraperitoneal

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* A preliminary report of work which has been aided by a grant from the Faculty Research Committee of the University of Pennsylvania.

[1] Katzman and Doisy, *J. B. C.*, 1932, **98**, 739.

TABLE I.
Results Obtained with Injections of Alfalfa Extracts.†
The dosage is expressed in terms of the equivalent of dry, crude alfalfa meal, one kilo being the equivalent of one entire extract.

Duration of alkaline extraction hr.	Extract	Dose in gm.	Ovarian response
4	1	400	+
	1	600	+
	2	750	+
	3	400	+
	3	300	+
	3	300	+
	2	250	—
24	4	750	—
	5	500	—
	5	500	—
	6	500	—
	6	400	—
	7	900	—
	8	800	—
	9	600	—

† The successful extracts were prepared from 3 separate shipments of alfalfa which differed considerably in color, odor, texture, and protein content.

injections of a variety of materials, including male urine and sterol suspensions, serve adequately as controls.

It is apparent, then, that gonad-stimulating extracts can be prepared from alfalfa. The active material may, or may not, be related to the pituitary hormone, and these data may or may not have direct physiological significance, although they are in harmony with the working hypothesis which stimulated this work.

7345 P

Experimental Studies in So-called Agranulocytic Angina. I. Effects upon Leucocytes by Toxic Products of Bacteria from Stool of Human Case.

WILLIAM H. HARRIS AND HERBERT J. SCHATTENBERG.

*From the Department of Pathology, School of Medicine, Tulane University.**

The possible causal relationship of the intestinal bacterial flora in agranulocytic angina has not been investigated. We carried out bacteriological examinations of stool specimens of a human case and recovered together with other of the intestinal microorganisms, cultures of *B. enteritidis*, *Streptococcus hemolyticus* and *B. welchii*. This paper deals with the results of animal inoculation with these cultures.

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* Aided by a grant from the David Trautman Schwartz Research Fund of Tulane University.

B. enteritidis. Six guinea pigs were injected, 2 subcutaneously, 2 intraperitoneally, and 2 intracardially, with 1 cc. each, of a saline suspension of this strain. Neutrophilic leucocytosis resulted and the animals died in 24 to 72 hours.

Three guinea pigs were fed with oats contaminated with stools containing *B. enteritidis*, and over the food of 3 guinea pigs suspensions of recovered culture were poured. After 6 weeks, including repeated leucocytic counts, no appreciable effects of such feedings were noted.

Two guinea pigs were injected, 1 intracardially and 1 intraperitoneally with the filtrate of broth cultures and increase of total and granulocytic leucocytes resulted.

In previous work upon production of lesions of experimental typhoid fever in the guinea pig with *in vivo* prepared toxic products of *B. typhosus*,[1, 2] it had been noted that a marked leukopenia resulted. No observations upon the granulocytes were made. We employed this method in the present work. A fatal peritonitis is primarily produced and the resultant exudative materials including toxins and saline washings of the peritoneal cavity were collected and filtered through a Berkefeld or Seitz-Wertz filter. A modified toxic moiety is procurable. Fifteen guinea pigs were injected by this method, employing the *B. enteritidis* isolation. The toxic filtrate was administered in doses of 1 to 5 cc. subcutaneously, intraperitoneally and intracardially. The intraperitoneal and intracardial routes and the larger doses were more effective. In all animals, a distinct lowering of the total leucocytic counts and a proportionately greater reduction of the granulocytes were observed. The period at which the greatest decline occurred averaged 72 hours with ordinarily an increment in the first 24 hours. The count of 1 animal injected intracardially is seen in the chart. Reductions of from 57% to 73% of the total and of from 90% to 96% of the granulocytes occurred.

Streptococcus hemolyticus. The inoculation of this culture and of filtrates of the broth culture subcutaneously, intraperitoneally and intracardially into guinea pigs yielded neutrophilic leucocytosis. *In vivo* prepared toxic products were obtained by the intraperitoneal

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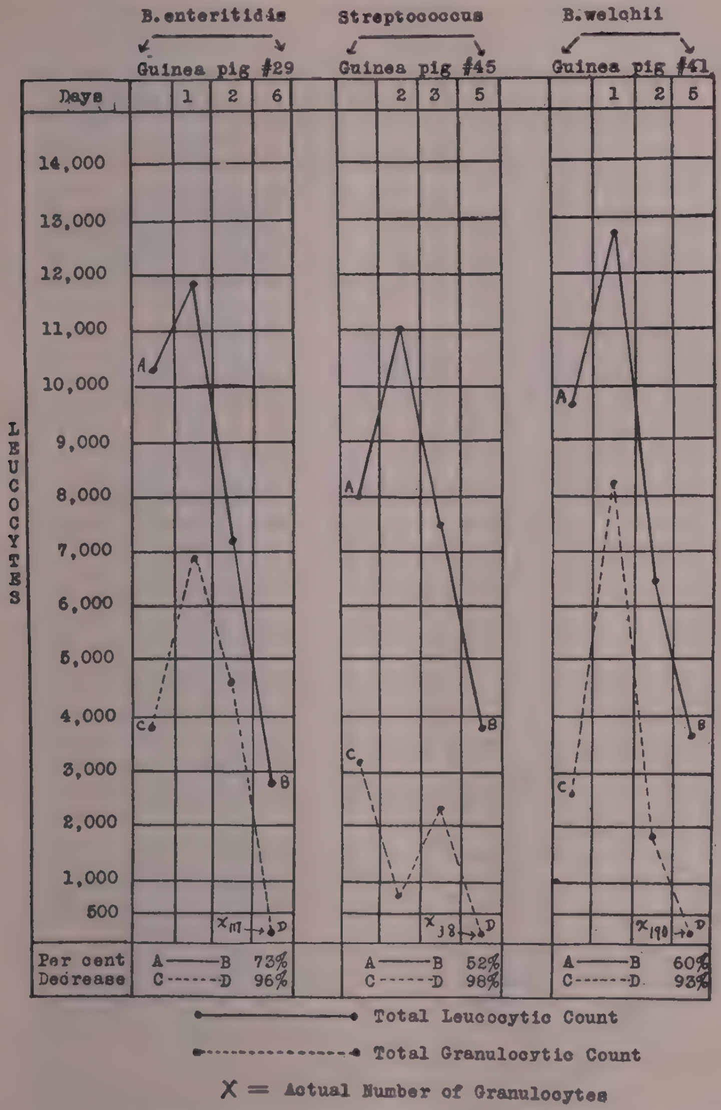
[1] Harris, W. H., PROC. SOC. EXP. BIOL. AND MED., 1928, **25**, 372.

[2] Harris, W. H., and Larimore, O. M., *J. Exp. Med.*, 1928, **48**, 885.

CHART 1.
Effects of Bacterial Toxic Products upon Leucocytes.



injection of this culture into the guinea pig. The Seitz-Wertz filtrate of the peritoneal exudate and washings was injected intraperitoneally into 2 guinea pigs, 45 and 46 in doses of 5 cc., and intracardially into guinea pigs 43 and 44, each receiving 3 cc. Considerable reduction of the total leucocytic count with marked reduction of the granulocytes was produced (see chart).

B. welchii. The whey portion of the litmus milk cultures was filtered through a Seitz-Wertz filter. This filtrate in amounts of from 2 to 10 cc. was introduced daily for 2 weeks, through a tube, into the stomach of 6 rabbits. No effect upon the general well being of these animals or upon the leucocytic counts was noted. The introduction of the living culture in this manner into 4 rabbits showed no change. This toxic filtrate of the whey portion of the culture was administered into 2 rabbits intravenously and 9 guinea pigs intracardially. Each rabbit was given 2 doses intravenously of 1 cc. at 6-day intervals. The total leucocytic count was usually increased following these injections, with a corresponding increase of the granulocytes but after 72 hours, the total count returned to normal. The granulocytes, however, were reduced from an average of approximately 40% to amounts as low as 5% following a primary rise but later returned to normal. The guinea pigs injected intracardially with this toxic filtrate in doses of more than 1 cc. succumbed within 24 hours. In the animals inoculated with 0.5 cc., the leucocytic and granulocytic curves were the same as in the rabbit, *i. e.*, primary rise of both with subsequent return of the total count but diminution of the granulocytes to between 5 and 8%.

Four guinea pigs were injected intraperitoneally with 3 to 5 cc. of the *B. welchii* filtrate and these animals yielded marked reductions of both the total and granulocytic counts. The leucocytic findings of a representative animal are shown in the chart.

It is to be noted that for this culture the *in vitro* toxin was sufficient to produce the leucocytic depressions and that no *in vivo* prepared toxic material was necessary.

Repetition of the injections of the toxic factors of these microorganisms that produce granulocytopenic leukopenia, lead to lowered resistance and death of the animals in 2 to 4 weeks, often from secondary invaders; this commonly occurs in the human case.

It is shown in these experiments that the *in vivo* prepared toxin of *B. enteritidis* and *Streptococcus hemolyticus* and the *in vitro* toxic filtrate of *B. welchii* will produce the blood picture found in the human case of so-called agranulocytic angina.

7346 P

Experimental Studies in So-called Agranulocytic Angina. II. Effects upon Leucocytes by Toxic Products of Staphylococcus aureus from Blood of Human Case.

HERBERT J. SCHATTENBERG AND WILLIAM H. HARRIS.

*From the Department of Pathology, School of Medicine, Tulane University.**

The strain of *Staphylococcus aureus* employed was obtained from the blood stream of a fatal chronic human case of so-called agranulocytic angina 4 days before death. This patient presented boils and 2 large abscesses.

Injections of suspensions of this culture of *Staphylococcus aureus* subcutaneously into 2 guinea pigs and intraperitoneally into 2 other such animals produced neutrophilic leucocytosis.

Rabbits 2, 3, and 5 were inoculated in the following manner: Rabbit 2 was injected with 2 cc. of the living suspension in the peritonsillar structures. A marked inflammatory reaction occurred and resulted eventually in necrosis. Frequent leucocytic counts revealed only a neutrophilic leucocytosis. In Rabbit 3, the upper tracheal mucosa was scarified with a hypodermic needle and 1 cc. was injected in and about the eroded area. Neutrophilic leucocytosis ensued.

Rabbit 5 was injected subcutaneously with 5 cc. of melted nutrient agar. This was allowed to solidify and 0.5 cc. of the culture of *Staphylococcus aureus* was introduced into the agar mass. No reduction of the total or granulocytic cells resulted. The Seitz-Wertz filtrate of broth cultures of this microorganism produced only neutrophilic leucocytosis when injected into guinea pigs.

An *in vivo* prepared toxic filtrate was obtained by producing peritonitis in guinea pigs with this culture. A Seitz-Wertz filtrate of the exudative material and peritoneal washings of these supply animals was inoculated into 6 guinea pigs, 3 intracardially and 3 intraperitoneally. Considerable reductions of the total counts and marked granulocytopenia was produced in these animals (see chart). It is of interest to note that one of these animals developed an erythroblastic anemia with 40,000 nucleated red cells per cu. mm., indicating a disturbance of this portion of the hemopoietic system as well as of the myeloblastic centers.

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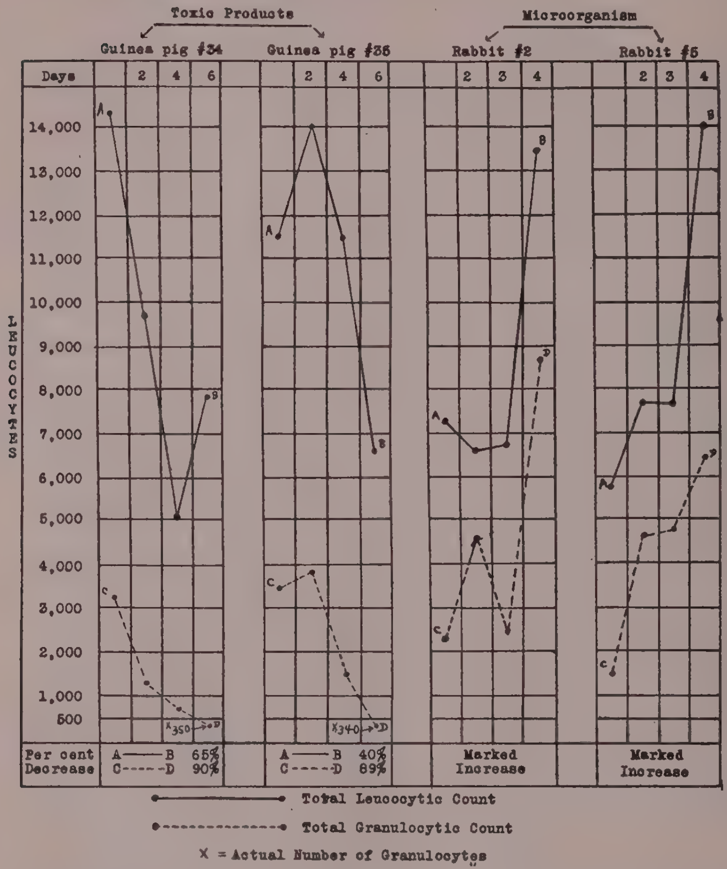
* Aided by a grant from the David Trautman Schwartz Research Fund of Tulane University.

CHART I.
Effects of *Staphylococcus aureus* upon Leucocytes.



Dennis[1] has shown that when various virulent microorganisms are enclosed in parchment capsules and introduced into the peritoneal cavity of rabbits, in some instances reduction of the total leucocytes and granulocytes is produced by the permeating toxins.

The *Staphylococcus aureus* is commonly found as an intercurrent infection in the human case. It is shown in these experiments that depression of leucocytes and of granulocytes is produced by the toxic moiety of this microorganism when obtained through an *in vivo* method.

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[1] Dennis, E. W., *J. Exp. Med.*, 1933, **57**, 993.

7347 C

Colored Flocculation Reactions.*

HUGO HECHT. (Introduced by B. S. Kline.)

All flocculation reactions are based upon colloidal chemical processes. What is more natural than to introduce into these reactions dye colloids for the purpose of making the flocculation more distinct, firstly by coloring the flocculi and secondly by making them more voluminous by the joining of the dye colloids in the flocculation of the extract.

Experiments of this kind have been made frequently, but have been only partially successful.

Experiments were reported[1] in which the extract colloid could be colored, whereas a contrast coloration was hardly possible of attainment. These experiments were carried out on the Hecht Flocculation Reaction (H. F. R.), on the Sachs-Georgi-Reaction (S. G. R.), and the typhoid reaction, using the Flicker Diagnostikum.

The experiments since then, carried out with well over 100 of the most diversified dyes, led to a provisional result as follows:

The goal of these studies was the development of a flocculation reaction in which a part of the coloring matter would join with the flocculated extract so that in the case of a positive reaction the fluid remaining after the flocculation of the extract would be colored differently than the fluid in a negative reaction (with the extract not flocculated).

Experiments of this sort were done particularly with the Hecht-Müller-Ballung reaction but show in their results that they may be employed in all flocculation reactions for syphilis which are done with alcoholic extracts without addition of Balsam (Sachs-Georgi-Reaction, Kahn Reaction, Kline Reaction, Hinton Reaction, etc.).

The principle of the staining consists in the following: 1. The extract is electively stained by a dye which does not leave any color in the fluid remaining after the precipitation of the extract colloid. 2. As counterstain of the salt solution (the extract-free fluid), dyes must be used which leave the extract colloid unstained or which color it but slightly. Among numerous lipoid stains, the only one of value was Sudan 3 of the I. G. Farbenindustrie.

The stain concentration of the extract is determined by a simple titration. In a wide-mouthed bottle that has been filled with the extract referred to, a tip of the knife full of Sudan red is added, the mixture well shaken and allowed to settle (it may also be centrifuged). A *saturated* stained extract results. Then with some positive and negative sera, the desired reaction, in our case the Hecht-Müller Ballung Reaction with the routine technic is done, using the undiluted colored extract and also dilutions of colored and uncolored extract in various proportions as follows:

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* Extract of report May 11, 1933, at the Alfred Fournier Institute in Paris.

[1] Hecht, *Med. Klinik*, 1922, **14**.

	cc.	cc.	cc.	cc.
Colored Extract	5	4	3	2
Uncolored Extract	1	1	1	1

A simple orientation experiment following the outline above will show the dilution in which *just that amount of dye is present in the extract that will be taken up completely in the balls of the positive test* so that the fluid is perfectly or almost perfectly clear, whereas in non-flocculated extract (negative reaction) a reddish, definitely different fluid is observable.

For counterstaining one may use dyes of many kinds, which must not be fat soluble. I recommend as stock solutions Löffler's methylene blue, 1% watery victoria blue solution, ½% watery naphthol green solution, etc. Here also the following principle applies: *The colored salt solution should contain only that much counterstain which, on mixture with the colored extract, does not permit recognition of its own color.* In a positive reaction, the red colored extract colloid is concentrated in the balls, and the counterstain is clearly visible whereas in the negative reaction a very indefinite mixed color of the turbid fluid is observed.

Examples. The cholesterin-free extract with which these experiments were done has its optimum action in a mixture of 3 cc. saturated stained extract and 1 cc. of unstained extract.

As counterstains, the following are satisfactory: 10% Löffler's methylene blue, (orig. alk.) 0.20 cc. to 20 cc. salt solution; 0.1% victoria blue, 0.1 cc. to 10 cc. salt solution; ½% naphthol green, 0.2 cc. to 30 cc. salt solution.

One can also prepare a combination of several counterstains giving a very good background for the scarlet red ball that forms with positive serum. The above mentioned stains give a good combination in the following mixture: Methylene blue, 1 drop to 40 cc., 3 parts; 1% victoria blue, 1 drop to 35 cc., 3 parts; ½% naphthol green, 1 drop to 15 cc., 4 parts.

Technique of the colored Hecht-Müller Ballung Reaction (H. M. F. B. R.). Performance of the colored Ballung Reaction:

Stable stock solutions: 1. 10% salt solution. 2. Slightly alkaline salt solution: NaCl, 0.9; NaOH, 0.1; Aqua destill ad 100.

3. Stains: Löffler's methylene blue, ½% naphthol green, 1% watery victoria blue solutions are stable. From these stains one prepares the proper quantity before each experiment. 10% methylene blue, 0.1 cc. to 30 cc. salt solution; 0.1% victoria blue, 0.1 cc. to 10 cc. salt solution; ½% naphthol green, 0.20 cc. to 30 cc. salt solution.

Every day the required salt solution is *freshly* prepared from stock solutions 1 and 2. 8 cc. of 10% salt solution, add 0.8 cc. of alkalinized salt solution, make up to 100 cc. with distilled water. 0.25 cc. of inactivated serum is employed.

The ready made colored extract which may be procured from the author is diluted as follows:† 1 cc. of extract and 0.55 cc. fresh salt solution as prescribed above are whirled about several times in a small wide-mouthed bottle. After a ripening period of 10 minutes, 0.6 cc. salt solution are added thereto and mixed again by whirling about. 0.15 cc. of this extract dilution is promptly added to the previously prepared serum. The rack is then shaken vigorously a number of times and then 1.5 cc. of the properly selected counterstain salt solution mixture is added. Further shaking is not necessary.

The rack is placed in a water bath at 55 or 56° and is removed after 10 minutes. One half hour later the first reading is made. One and a half hours later, the final one is made.

The reading of the results is very easy. When naphthol green has been employed as the counterstain, the strongly positive serum shows a large bright red ball floating in clear green fluid. The ball gradually sinks to the bottom in the following hours. The negative sera have a cloudy reddish color.

Appearance of small red colored flocks, especially more than 1½ hours after the conclusion of the experiment is not to be considered as indicating a positive test.

Even 12 hours later, the difference between positive and negative tests is still readily observable.

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† This extract, a description of which was published in 1921, is prepared as follows:

To a weighed amount of powdered, dried fat-free beef heart add twice the weight of ether. Extract once during a short period. Evaporate to dryness and weigh the residue. Add 5 times the weight of 96% alcohol and place this for 48 hours in an incubator at 56° C. Cool the extract and filter.

When Löffler's methylene blue solution is used, the strongly positive sera are characterized as described above by a bright red ball and this is observed floating in clear blue-green fluid. The negative sera are cloudy, reddish.

With victoria blue as a counterstain, a large violet ball forms in positive serum and floats in clear blue fluid. The negative sera are cloudy, violet.

If it is desired to intensify the colors, what was said in the introduction must be borne in mind. The intensification must be made in such a fashion that both the red color of the extract and the counterstain of the salt solution are intensified in a certain proportion. If more red colored extract is used, more serum must be employed and more salt solution to make up the mixture to the proper quantity.

Accordingly, if instead of the standard quantity of diluted extract, a larger amount is used, for example 0.2 cc., then 0.35 cc. of serum is employed and the counterstain is intensified. In such case one takes of stock solution of naphthol green, 0.25 cc. to 30 cc. salt solution, of methylene blue 0.10 to 21.5 cc. salt solution, and of victoria blue solution 0.1 to 75 cc. salt solution.

Anyone who has worked for some time with colored extracts will never care to work otherwise, since the readings with them are easy and simple.

Summary. A method is suggested for all flocculation reactions, especially for the Hecht-Müller Ballung Reaction of elective staining of the extract colloid which brings about a distinctive color effect that facilitates the reading of results without impairing their specificity. Readings are best made against a white background (white paper). Positively reacting sera show a bright red ball, and after it is formed the chosen counterstain, blue, violet, or green appears in the surrounding fluid. Negative sera show a turbid light violet to greenish-blue color, permitting an immediate differentiation.

7348 P

Cardiac Output in the Pick Syndrome.

ALICE B. MALTBY. (Introduced by C. S. Beck.)

From the Laboratory of Surgical Research, School of Medicine, Western Reserve University, Cleveland.

The cardiac output of patients with the Pick syndrome was determined by means of the Grollman[1] indirect acetylene method. This procedure is applicable to such patients in spite of their reduced vital capacity if the volume of the gas mixture to be rebreathed is decreased and if the rebreathing period is slightly increased.

The cardiac output per minute per square meter of body surface Grollman[1] calls the cardiac index. He has found that under basal conditions the normal person has a cardiac index of 2.2 ± 0.3 liters per minute. Any deviation of the index from the normal 2.2 greater than 15%, when determined by the acetylene method, is considered pathological.

Mrs. S., age 54 years, with Pick's disease, who should have had an output of 3.50 liters per minute (calculated by multiplying the patient's surface area by 2.2), had an average output of 2.26 liters per minute, 36% below normal. The estimated normal output per beat is 47 cc.; she had an average beat output of 20 cc., her heart rate being 114 per minute.

Mrs. M., age 32 years, was operated on for the Pick syndrome. Her output normally should have been 3.04 liters per minute. Before operation she had an average minute output of 1.79 liters, 41% below normal. The tests after operation were carried out over a 2 weeks' period starting a month and a half after operation. Although there was not a striking increase in the average minute output of 1.84 liters (39% below normal), 5 of the 12 tests after operation reached a value never attained before operation. An average of the best 6 tests after operation gives an output of 2.05 liters per minute, only 33% below normal. This tendency toward improvement indicates that her heart has a reserve power and leads to the belief that as time goes on this patient's output will continue to improve. Before operation her beat output averaged 24 cc., her average heart rate being 75 per minute. After operation her beat output averaged 26 cc., the average heart rate being 71 per minute.

Mr. R., age 28 years, had a computed normal value of 4.25 liters

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[1] Grollman, A., *The Cardiac Output of Man in Health and Disease*, Charles C. Thomas, Baltimore, 1932.

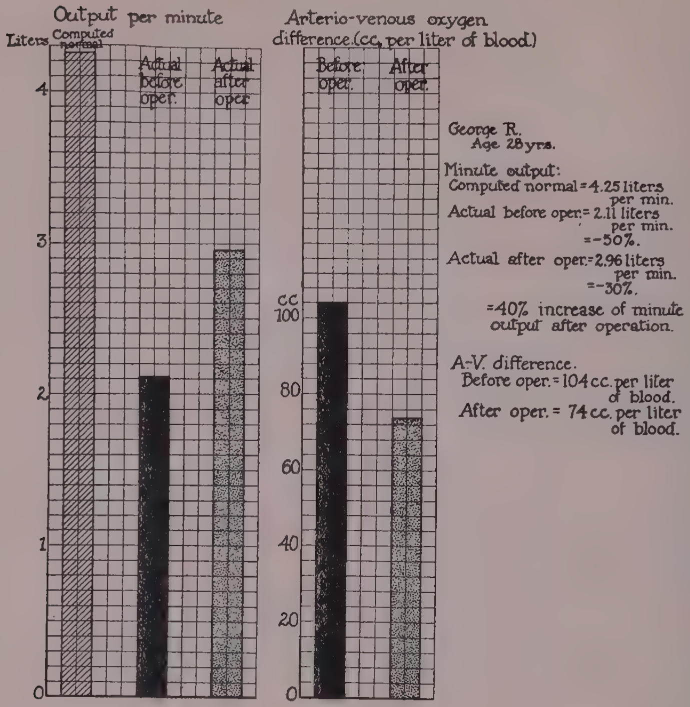

FIG. 1.

per minute. Before operation his average output was 2.11 liters per minute, 50% below normal. Six weeks after operation the output averaged 2.96 liters per minute, 40% above the preoperation output and only 30% below normal. (See chart.) In 3 of the tests after operation the output was practically normal *i. e.,* only about 15% below the computed normal value. Before operation his beat output averaged 28 cc.; his heart rate averaged 75 per minute. After operation his beat output averaged 34 cc.; his heart rate averaged 86 per minute.

The tests on F., a 17-year-old boy, are particularly interesting because they indicate the impovement of the patient 3 years after operation. (Unfortunately it was not feasible to get cardiac output tests on this boy before operation.) His computed normal output was 3.74 liters per minute and his average actual output of 3.27 liters per minute was only 12% below the computed normal value and therefore within the accepted normal range. His computed normal beat output was 53 cc. and his actual beat output of 46 cc. was only 13% less than his computed normal value and therefore within the accepted normal range.

7349 C

Urinary Excretion of Vitamin C.

ALFRED F. HESS* AND HELEN RIVKIN BENJAMIN.

From the Department of Pathology, College of Physicians and Surgeons, Columbia University.

Many authors have demonstrated that vitamin C exists in various organs and fluids of the body. Moreover, it has been shown that in the guinea pig the concentration of vitamin C in the body is dependent upon the intake,[1-4] and that in the tissues of the guinea pig the vitamin C concentration is lower than in species which are not dependent upon a dietary source of this vitamin.[3] In view of such evidence, it was thought worth while to investigate the urinary excretion of vitamin C. The experiments were planned to throw light upon the following questions: 1. Is vitamin C excreted by the urinary route? 2. Is the level of urinary excretion proportional to the intake or is it a measure of excess vitamin ingestion? 3. Is there a difference in urinary excretion between species which do and those which do not require a dietary source of the vitamin?

As far as we are aware, very little work on this subject has been reported. von Euler and Malm[5] state that they were unable to detect vitamin C in normal, febrile or diabetic urine. On the other hand, van Eekelen and his associates[6] found that urinary excretion ran parallel to intake, but since these authors do not give any figures, no comparison between species can be made on the basis of their work.

METHOD

The method employed was that of Birch, Harris and Ray,[7] based on the titration of ascorbic acid against 2:6 dichlorophenolindophenol. Since urine is known to contain only traces of the compounds (glutathione and other sulphydryl compounds) which form the chief source of error in the application of this method, it seemed safe to consider any reducing substance found in urine to be ascorbic acid.

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* This work was completed after the death of Dr. Alfred F. Hess.

[1] Svirbely, L. J., and Szent-Györgyi, A., *Biochem. J.*, 1933, **27**, 279.

[2] Harris, L. J., and Ray, S. N., *Biochem. J.*, 1933, **27**, 303.

[3] Bessey, O. A., and King, C. G., *J. Biol. Chem.*, 1933, **103**, 687.

[4] Siehrs, A. E., and Miller, C. O., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 696.

[5] von Euler, H., and Malm, M., *Arch. f. Kemi. Min. Geol.*, 1932, IIB, 6, 7, 8, 9.

[6] van Eekelen, M., Emmerie, A., Josephy, B., and Wolff, L. K., *Nature*, 1933, **132**, 315.

[7] Birch, L. W., Harris, L. J., and Ray, S. N. *Biochem. J.*, 1933, **27**, 590.

The dye solution was freshly prepared every second day and was standardized against a CCl_3COOH solution of ascorbic acid. Through the kindness of Dr. Szent-Györgyi, the latter was supplied to us in the form of the commercial preparation "cevita". This was found to be an exceedingly convenient source, since it consists of tablets, each of which contains 5 mg. ascorbic acid. The accuracy of these was checked periodically against some pure crystalline ascorbic acid which Dr. King was good enough to give to us.

Since vitamin C was found to be exceedingly unstable in alkaline solution† the urine was collected in bottles containing several drops of glacial CH_3COOH. The urine was then mixed with 20% CCl_3COOH in proportions to make the final concentration of the latter about 5%, filtered and titrated, usually against 0.02 cc. dye solution. Since the urine solution was always colored, it was necessary, in carrying out the titration, to use 2 similar tubes, one containing dye and the other a blank, containing only urine, to the color of which the end point was matched.

This method proved to be highly satisfactory for urines containing large amounts of vitamin C, but was very much less accurate for the more dilute specimens. Attempts to concentrate the vitamin by evaporation of the urine or by precipitation of the ascorbic acid with basic lead acetate, led to losses in potency and were abandoned. All of the urine, CCl_3COOH filtrates, including the dilute ones, were, therefore, used directly for titration. The average was taken of 3 to 6 titrations on each urine. Since the differences here reported, which were produced by administration of vitamin C, are of the order of 1000%, the relative lack of accuracy, even in the most dilute urines (a maximum of about 50%), is not sufficient to interfere with the interpretation.

When ascorbic acid was added *in vitro* to CCl_3COOH urine filtrates, it could be recovered to the extent of 78 to 105% (av. 87%). The more concentrated the solution, the more complete was the recovery.

EXPERIMENTS ON CHILDREN

The experiments were divided into several periods representing different levels of vitamin C intake. The urine was collected in 24-hour samples and examined daily.

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† If the solution was made faintly alkaline, its reducing power was completely lost after standing overnight in the icebox. This was true of urine as well as of solutions of pure ascorbic acid and of orange juice. Reacidification at this point did not restore the reducing power.

In Table I, a typical record is presented on one of the 7 children studied. It is at once apparent that under ordinary dietary conditions, the amount of vitamin C present in human urine is so small as to be considered entirely negligible. The concentration was about one-fortieth of the lowest value found by Bessey and King[3] in a human organ (heart). This was true whether the vitamin was entirely excluded from the diet or whether it was fed in adequate amounts, either in natural foods (1 oz. orange juice daily and the usual amounts of fruits and vegetables) or as pure ascorbic acid (5 "cevita" tablets daily, containing 25 mg. ascorbic acid or the equivalent of about 1½ oz. orange juice). The level of urinary excretion of vitamin C, therefore, appears not to be proportional to the dietary supply. Only when excessively large doses of vitamin C were fed (1 pt. orange juice daily) did appreciable urinary excretion occur. Two characteristic features appear in the table in connection with the rise. It did not occur until the fourth day of high vitamin feeding, and when it did occur, the increase was abrupt and very marked. The highest concentration was usually reached on the fifth day, after which a slight reduction often occurred in spite of

TABLE I.

Effect of Vitamin C Feeding on Urinary Excretion in a Child.

S. B.—3 years old—31½ lb.

Date	Vitamin C Fed*	Ascorbic Acid in Urine	
		per cc.	per 24 hr.
		mg.	mg.
1-12	Normal amount	.001	—
13	" "	.002	.55
14	" "	.002	.84
15	None	.002	1.08
16	"	.002	1.15
17	"	.001	—
18	1 pt. orange juice	.003	2.18
19	" " "	.001	—
20	" " "	.003	2.13
21	" " "	**.023**	**13.31**
22	" " "	.101	58.90
23 24	" " "	.032	15.50
25	" " "	.050	30.70
26	None	**.002**	**1.18**
27	1 pt. orange juice	**.045**	**18.38**
28	" " "	.031	14.05
29	" " "	.021	16.01
31	" " "	.062	49.40
2-1	" " "	.085	60.80

* Fed during 24 hrs. preceding collection of urine.

continued feeding. The highest value which we observed in this study was 0.188 mg. ascorbic acid per cc. urine or a total excretion of 115 mg. in 24 hours. The intake, in a pint of orange juice, was approximately 275 mg. ascorbic acid.

When the vitamin C feeding was stopped, an immediate reduction in urinary excretion must have occurred, since in the sample for which the collection was begun 24 hours after the last feeding, the concentration of ascorbic acid had already returned to the original negligible level.‡

This observation, coupled with that of the initial lag in urinary excretion following high dosage, and with the finding of negligible amounts of vitamin C in the urine when smaller, but adequate amounts of the vitamin were fed, suggest the explanation that vitamin C in the urine represents an excess which appears only after complete saturation of the tissues has occurred. This hypothesis is also supported by further data obtained on 3 children. In these, after the level of urinary excretion of vitamin C had reached a high value, the orange juice feeding was stopped for only one day and then resumed. The result may be seen in the table (2/26 and 2/27). An immediate rise in urinary excretion followed the resumption of the orange juice feeding. It would appear, therefore, that when the tissues are thoroughly saturated with vitamin C, no lag occurs in the urinary response to feeding of large amounts of the vitamin.

Of the 7 children studied, 2 deviated from the typical course outlined above. In one of these, after a pint of orange juice had been fed daily for 7 days, the urinary content of vitamin C was still not increased. The experiment was stopped at this point because the child became ill with an infection. In the second atypical case, the level of urinary excretion rose as usual, 4 days after the orange juice feeding was begun. When the orange juice was stopped, the drop in urinary excretion was gradual, requiring 3 days to return completely to the "normal" level. Orange juice feeding was resumed at this point and the rise in urinary excretion of the vitamin was similarly gradual, taking 2 days to appear and continuing to mount for the next 3 days. In this instance, it is probable that the continued excretion of the vitamin, after the feeding was discontinued, led to a depletion of the tissue stores, which were first replenished before the excess vitamin appeared in the urine.

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‡ In instances in which this period of the experiment was prolonged, the urinary vitamin C remained at the same low level.

EXPERIMENTS ON OTHER SPECIES

Similar studies were also made on guinea pigs, rabbits and rats. The orange juice was fed by pipette to the guinea pigs, and rats and was given by stomach tube to the rabbits. These 3 species showed identical reactions, as regards urinary excretion of vitamin C, which, however, differed from those exhibited in the human series. The data are summarized in Table II. When no vitamin C was fed, the initial concentration in the urine of guinea pigs, rabbits and rats was approximately the same (about 5 to 10 times as high as that in human urine) and the total excretion in 24 hours was proportional to the total volume of urine. Moreover, even when very large doses of vitamin C were fed, no corresponding increase in urinary concentration resulted. This was true when the vitamin C was fed as orange juice; as orange juice + HCl (to insure acidity of the urinary tract); or as ascorbic acid ("cevita" in H_2O or in acid). As regards the urinary excretion of vitamin C, it would, therefore, appear that the human species is different from the others studied, but that no distinction exists between species (guinea pig and human) which do, and those (rabbit and rat) which do not, require a dietary source of the vitamin.

In the case of the guinea pig, no difference was observed between normal animals and those which had moderate scurvy, either as to initial vitamin C concentration in the urine, or urinary response to vitamin C ingestion.

TABLE II.
Effect of Vitamin C Feeding on Urinary Excretion in Various Species.

Species	Daily Intake Vitamin C	Aver. Ascorbic Acid in Urine		No. Determinations
		per cc.	per 24 hr.	
		mg.	mg.	
Guinea pig	None (oats)	.014 ± .0010*	.146 ± .0400*	13
	Normal (carrots, lettuce)	.007 ± .0006*	.300 ± .0237*	16
	Excessive (10-60 cc. orange juice)	.005 ± .0005*	.167 ± .0579*	23
Rabbit	None ("Purina Chow")	.021 ± .0023*	1.950 ± .0607*	18
	Excessive (10-80 cc. orange juice)	.034 ± .0033*	2.670 ± .2630*	18
Rat	None (McCollum Diet)	.012 ± .0026*	.147 ± .0427*	7
	Excessive (10-20 cc. orange juice)	.010 ± .0009*	.062 ± .0084*	8
Human (Children)	None (No fruits or vegetables)	.002 ± .0002*	1.27 ± .1300*	29
	Normal (1 oz. orange juice)	.002 ± .0003*	.984 ± .1017*	21
	Excessive (1 pt. orange juice)	.058 ± .0066*	26.23 ± 2.84*	41

* Probable error.

When vitamin C in large quantities similar to those which had been fed (6 to 8 "cevita" tablets in H_2O) was injected intraperitoneally into guinea pigs, or rabbits, a sharp rise (3 to 20 fold) in urinary excretion occurred, usually on the second or third day of injection. When the injections were stopped, the reduction in urinary concentration was often quite gradual. These experiments suggest that in these species a certain amount of destruction of vitamin C occurs in the digestive tract, or that the degree of intestinal absorption of the vitamin is limited.

Conclusions. It would appear that under ordinary nutritional conditions, vitamin C is not excreted in appreciable amounts in human urine and is excreted only in very low concentration in the urine of guinea pigs, rabbits and rats. In the human species (children) ingestion of excessively large amounts of vitamin C leads to urinary excretion after the body stores have become completely saturated. In the other species, urinary excretion can be raised only by injection of the vitamin. There is no difference in urinary excretion of vitamin C between species which do and those which do not require a dietary source of the vitamin.§

7350 P

Studies in the Repair of Bone.

WILBERT H. McGAW. (Introduced by Claude S. Beck.)

From the Laboratory of Surgical Research, the School of Medicine of Western Reserve University and the Lakeside Hospital.

The supremacy accorded the periosteum in osteogenesis has led to an almost complete neglect of the rôle played by other bone elements. Work on epiphyseal tissue has indeed been very scant, but there is both clinical and experimental evidence that cancellous bone plays an important rôle in bone repair, while the osteogenic properties of bone marrow and endosteum though often mentioned have been little studied.

Epiphyseal Tissue Transplants. A set of experiments were carried out on 6 immature dogs. A quarter inch extraperiosteal resection of each fibula was filled on one side with ground epiphyseal tissue while on the control side the defect was filled with ground whole bone. The defects were bridged across in each case. The epiphyseal tissue invariably produced a large thick spongy callus. Serial slides of X-rays were presented to illustrate this callus at different stages of development, Fig. 1.

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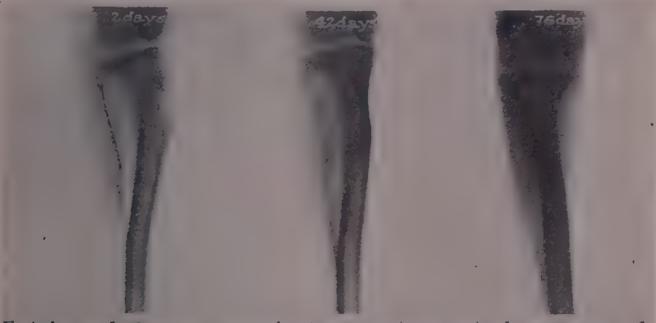
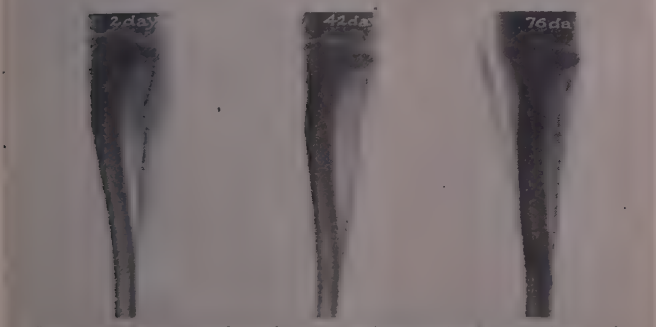
§ Since the completion of this paper, an article has appeared by L. J. Harris, S. N. Ray, and A. Ward in the *Biochemical Journal* (Volume 27, page 2011) entitled "The Excretion of Vitamin C in Human Urine and Its Dependence on the Dietary Intake". The authors conclude that in adult urine after the ingestion of a single very large dose of orange juice, the urinary excretion of vitamin C rises to a maximum which is reached after three hours.

Cancellous Bone Transplants. Experiments were done on 5 adult dogs. Cancellous bone was removed from the condyle of the femur and transplanted into extraperiosteal and subperiosteal defects of the 2 fibulae respectively on each dog. These defects bridged across in each case even where there was no existing periosteum. Slides of



Epiphyseal tissue transplant into subperiosteal resection of the right fibula.



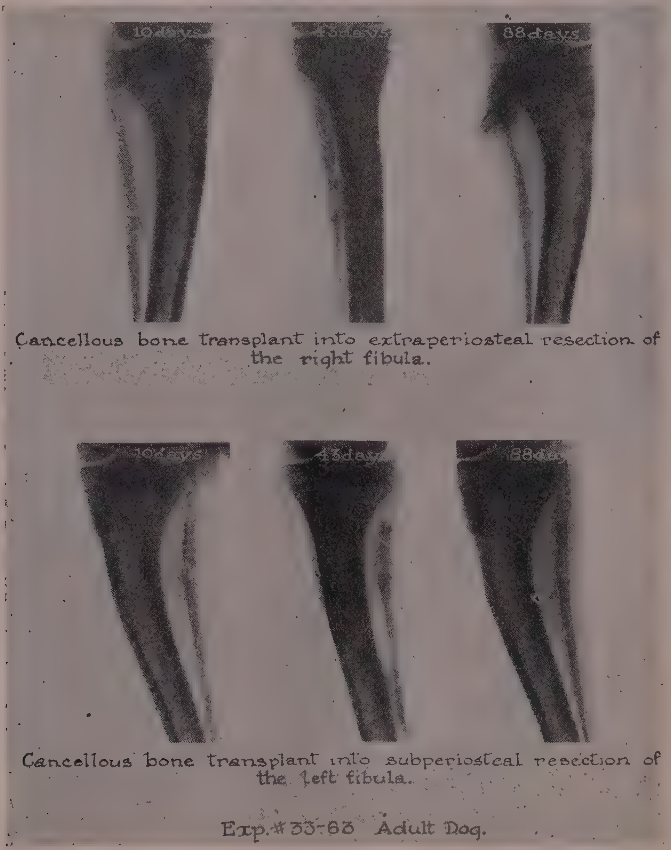
Cortical bone replaced into subperiosteal resection of the left fibula for control.

Exp.#179. Dog, age approximately 10 months.

FIG. 1.

serial X-rays were presented to illustrate how rapidly cancellous bone grafts regenerate new bone. (Fig. 2.) On the side of the subperiosteal resections the favorable presence of intact periosteum is well illustrated in a more rapid regeneration.

Bone Marrow and Endosteal Transplants. The next group of experiments was carried out on 6 adult dogs. Each fibula was resected extra-periosteally. On each side the defect was filled with endosteum and bone marrow removed from the tibia with a small curette. No regeneration occurred on the control side where no grafts were placed, while on the side containing endosteum and bone marrow rapid and complete reformation of dense bone oc-



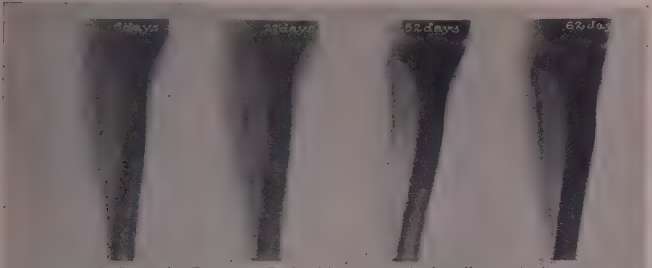
Cancellous bone transplant into extraperiosteal resection of the right fibula.

Cancellous bone transplant into subperiosteal resection of the left fibula.

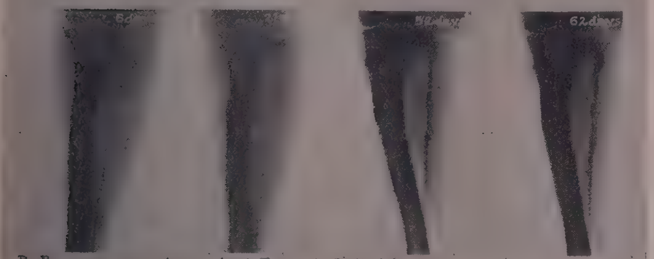
Exp. # 33-63 Adult Dog.

FIG. 2.

curred. Slides of serial X-rays were shown to illustrate this rapid osteogenesis. (Fig. 3a.) A photomicrograph was presented to show the new bone callus. (Fig. 3b.)



A. Control Exp.#19. Right fibula resected with periosteum.



B. Bone marrow transplant Exp.#19. Left fibula resected with periosteum.

FIG. 3a.

Conclusions. 1. Epiphyseal tissue will regenerate new bone in immature dogs. 2. Cancellous bone will bridge bony defects without the aid of periosteum. 3. Periosteum will hasten callus formation of cancellous bone grafts. 4. Free bone marrow and endosteum will bridge bone defects without the aid of periosteum with a rapid forming callus. 5. The clinical use of bone marrow and endosteum is suggested in bone grafting.

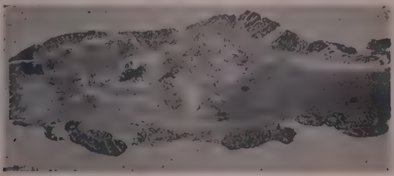


FIG. 3b.

7351 P

Artificial Production of the "Wedding Dress" in Chrosomus Erythrogaster.*

WILLIAM SAPHIR. (Introduced by W. Thalhimer.)

From the Nelson Morris Institute for Medical Research, Michael Reese Hospital, Chicago.

Recent reports[1, 2, 3] have made it probable that certain species of fish may be used successfully as test animals for the demonstration of either the gonadotropic or the sex hormones. For some time it has been known that certain male fish, such as *Rhodeus amarus, Gastrosteus, Phoxinus laevis,* belonging to the Cyprinidae group, assume striking color changes during the spawning period, lasting for several weeks in the spring. This so-called "wedding dress" or "mating coat" is due to the expansion of the chromatophoric cells of the skin and is considered to be an expression of heat in order to attract the females. Thus it would be comparable to the well-known estrus of the rodents.

Wunder,[1] working with *Rhodeus amarus,* obtained a "wedding dress" with subcutaneous injection of testis extract, variable results with prolan, and no effect with the female sex hormone. Glaser and Haempel[2] reported the castrated Bitterling as a suitable test animal for the demonstration of the male sex hormone. Zondek[3] used the expansion of the erythrophores of *Phoxinus laevis* as a test for the demonstration of his pituitary pigment hormone intermedin.

While these experiments were conducted on fish, domestic and very abundant in central Europe, they are not inhabitants of this country and are difficult to obtain. For this reason and for the purpose of producing the "wedding dress" artificially in an American fish, *Chrosomus erythrogaster* was considered the most suitable fish. This attractive and hardy fish, commonly called "red bellied dace", belongs to the group of Cyprinidae and is found in various parts of this country from Maine to Alabama in small, clear streams, where it breeds in May and June. No record exists of its having bred in

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* The author wishes to express his indebtedness to Dr. Wm. Thalhimer, Director of the Nelson Morris Institute, and also to Mr. W. Chute, Director of the Shedd Aquarium, Chicago, for their help and suggestions.

[1] Wunder, W., *Z. Wiss. Biol. Abt C, Z. Vergl. Physiol.,* 1931, **13**, 696; Abst., *Biol. Abst.,* 1932, **6**, 15908.

[2] Glaser and Haempel, *Deutsch Med. Wchnschr.,* 1932, **58**, 1247; *Endokrinologie,* 1932, **11**, 81.

[3] Zondek and Krohn, *Klin. Wchnschr.,* 1932, **11**, 405, 849, 1293.

captivity.[4] The breeding dress consists in a vivid and brilliant red discoloration of the otherwise white belly and the part just below the mandible on each side.[5]

As the experiments were undertaken in winter, I am unable to state whether the natural spawning habits of this fish in spring may interfere with the results of this study reported at this time.

The fish are kept in a common aquarium aerated with compressed air. The optimum temperature of the water is 50-60°F. For injection the fish is taken from the net and held between index finger and thumb of the left hand. The needle is introduced in midline between back fin and tail fin. The needle is kept superficially and pushed slightly forward. The amount injected is 0.1 cc. Care must be taken not to injure the air bladder which is followed by immediate death. This will be avoided by keeping the needle strictly in the midline and superficially. The deprival of water during this manipulation is well tolerated. The fish is placed thereafter in a glass bowl for the period of observation. The "wedding dress" becomes visible after 30 minutes and lasts from several hours to 24 hours or more.

Injections of physiologic saline solutions, urines of males, females, and of pregnant women, adrenalin solutions, female sex hormone (progynon and theelin), testis hormone, hebin (pituitary extract, Wilson), antuitrin (Parke Davis) were not followed by any visible changes. A fully developed brilliant wedding dress was rapidly and regularly produced by Yohimbine hydrochlor. 1:10,000, as also was noted in other species of fish by other investigators.[1, 2, 3] Prolan, prepared from the urine of pregnant women by Zondek's alcohol precipitation method,[6] acted inconstantly. While the percentage between positive and negative reactions amounted to about 50% with a urine extract concentrated 1:5, the impression was gained that the more prolan excreted by the patient, the more likely a positive reaction will result. Thus a fully developed mating coat was regularly obtained with a urine extract of a case of chorionepithelioma. The quantitative analysis of this urine revealed the presence of 333,000 mouse units of prolan per liter, while in normal pregnancy the amount of prolan excreted would not exceed 20,000 m.u. per liter. Further study along these lines is contemplated.

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[4] Mellen, Fishes in the Home. New York, 1927.

[5] Smith, Bertram G., The Spawning Habits of *Chrosomus erythrogaster Rafinesque, Biol. Bull.*, Woods Hole, 1908, **15**, 9.

[6] Zondek, B., *Die Hormone des Ovariums und des Hypophysenvorderlappens*, Julius Springer, Berlin, 1931.

Summary and Conclusions. While the results are not complete enough to reach definite conclusions, it would seem that in view of similar and better known conditions in rodents the mechanism of the production of the "wedding dress" in *Chrosomus erythrogaster* is due to the action of substances having either a stimulating effect upon the sex glands of the fish, such as prolan, or having an effect similar to that of the estrus producing hormones, such as Yohimbine.

7352 C

Effect of Prolonged Feeding of Raw Carrots on Vitamin A Content of Liver and Kidneys in the Dog.

R. G. TURNER.

From the Department of Medical Research, Wayne University, College of Medicine.*

The function of carotene in nutrition has been established.[1-4] The advisability of feeding large amounts of raw carrots over a prolonged period of time is still questioned. Carotinemia has been observed in the dog,[5] and in man.[6] Investigations of Moore,[2] Capper,[7] and Drummond[8] have shown that conversion of carotene to vitamin A takes place in the livers of vitamin A-free rats or fowls. Olcott and McCann[9] later confirmed these observations and further concluded that this change may take place *in vitro* if incubated with fresh liver tissue. Rea and Drummond[10] failed to obtain a carotinase in liver preparations from vitamin A-free rats and further obtained no increase in vitamin A content of cat livers after carotene administration.

Table I presents the results obtained by feeding dogs fresh car-

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* Formerly Detroit College of Medicine and Surgery.

[1] Euler, Euler and Karrer, *Helv. Chim. Acta.*, 1928, **12**, 278.

[2] Moore, T., *Biochem. J.*, 1930, **24**, 692.

[3] Green, H. N., and Mellanby, E., *Brit. J. Exp. Path.*, 1930, **11**, 81.

[4] Turner, R. G., and Loew, E. R., *J. Inf. Dis.*, 1933, **52**, 102.

[5] Duhig, J. V., *Med. J. Australia,* 1931, **1**, 260.

[6] Curtis, A. C., and Kleinschmidt, E. E., *Ann. Internal Med.*, 1932, **6**, 751.

[7] Capper, N. S., *Biochem. J.*, 1930, **24**, 980.

[8] Drummond, J. C., Ahmad, B., and Morton, R. A., *J. Soc. Chem. Ind.*, 1930, **49**, 291 T.

[9] Olcott, H. S., and McCann, D. C., *J. Biol. Chem.*, 1931, **94**, 185.

[10] Rea, J. L., and Drummond, J. C., *Z. Vitaminforsch*, 1932, **1**, 177.

TABLE I.
Vitamin A Content of the Liver and Kidneys in the Dog.

		Animal Units of Vitamin A			
gm. Diet	Dog No.	Liver	Kidney	Total	Days on Diet
150 meat**	51	12.6	8.0	20.6	63
150 boiled rice†	52	14.4	4.0	18.4	63
	50	16.0	6.02	22.02	63
150 fresh carrots	1	11.0	—	—	240
	4	26.6	12.1	38.7	224
Aver.		16.1	7.53	24.93	
150 meat	54	4.03	1.01	5.04	15
150 boiled rice	55	4.52	—	—	49
Aver.		4.27	1.01	5.04	
150 meat					
150 boiled rice	5	15.5	7.9	23.4	240
	6	14.1	4.8	18.9	240
150 fresh carrots	3	16.6	—	—	96
10 cc. C.L.O.					
Aver.		15.4	6.35	21.1	

** Maro dog meat from Chappel Bros., Inc., Rockford, Illinois.

† Boiled rice was prepared by boiling 1,000 gm. rice in 2,000 cc. water to which was added 2% NaCl and 3% $(Ca)_3(PO_4)_2$.

The figures presented represent the amount of vitamin A per gram of tissue.

rots over a prolonged period of time (2 to 8 months). Five of the animals received a diet of meat, boiled rice and fresh carrots; 3 received a similar diet with the addition of 10 cc. of cod liver oil daily, and 2 were deprived of both carrots and cod liver oil. The 2 dogs receiving only meat and boiled rice succumbed at 15 and 49 days. Previous to this their appetite became sporadic, there was marked discharge from the nose and eyes and in all the symptoms were those of distemper. The remaining dogs continued to maintain or gain in weight and appear healthy and vigorous. They were killed and autopsied at varying times. No signs of carotinemia were observed in any of the dogs. The liver and kidneys were removed at necropsy and prepared for vitamin A estimation by the method of Moore.[2]

The colorimetric estimation was carried out by the method of Norris and Danielson.[11] The dilutions of the extracted tissue oils were made so that the readings were below 2 blue Lovibond units. Norris and Church[12] have reported that the Carr-Price reaction at this low color value gives results less than 10% in error and that the color curve for vitamin A more closely approaches a linear function. The results are presented in animal units of vitamin A per gram of tissue. According to Rosenheim[13] an animal unit is that amount of vitamin A which produces a color equivalent to 10 Lovibond standard blue units. This amount restores normal growth to rats on a diet otherwise free from vitamin A. Spectroscopic examination of the blue color formed by the antimony trichloride reaction on oils obtained from both kidney and livers showed a characteristic sharp band at 608-610 Mu with an indefinite diffused band at 330-328 Mu.

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[11] Norris, E. R., and Danielson, I. S., *J. Biol. Chem.*, 1929, **83**, 469.

[12] Norris, E. R., and Church, A. E., *J. Biol. Chem.*, 1930, **85**, 477.

In general the liver and kidneys from dogs living 63 to 240 days on a diet containing 150 gm. of fresh carrots showed approximately the same amount of vitamin A. In only one case, dog 4, was there a marked increase encountered. This dog remained on the diet for 224 days. The total vitamin content for both organs was 38.7 A. U. as compared to 22.02 A. U. found in dog 50 which is the highest result found after feeding for 63 days. The average vitamin A content for the carrot-fed dogs is 16.1 A. U. per gram of liver and 7.53 A. U. per gram of kidney. This is approximately 4 times the amount found in the same organs of dogs receiving no carrots (dogs 54 and 55). According to Moore[14] 16.5 A. U. is equivalent to 1 mg. of carotene. The 3 dogs receiving cod liver oil in addition to the 150 gm. of carrots showed no further increase in the vitamin A content of the 2 organs than encountered in the carrot-fed dogs, averaging 15.4 A. U. per gram of liver and 6.35 A. U. per gram of kidney.

Summary. The vitamin A content of the liver and kidneys of dogs fed fresh carrots is greater than that found in dogs on a meat and boiled rice diet. The level of the vitamin A concentration in the liver and kidneys is approximately 20 vitamin A animal units. Continued feeding of carrots or addition of cod liver oil apparently does not increase the vitamin A content above this level. The spectroscopic examination indicates that the carotene from the carrots was transformed to vitamin A.

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[13] Rosenheim, O., *Biochem. J.*, 1927, **21**, 1329.

[14] Moore, T., *The Lancet*, August 24th, 1929, 380.

7353 P

Studies of Incurable Rickets. I. Respective Rôle of the Local Factor and Vitamin D in Healing.

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From the Pediatric Research Laboratory, the Jewish Hospital, Brooklyn.

This communication reports a type of incurable rickets caused by the substitution of 3% $SrCO_3$ for 3% $CaCO_3$ in a rickets-producing diet.

Rats were divided into 2 groups. To the control group the rickets-producing diet containing 3% $CaCO_3$ was fed while the other group received 3% $SrCO_3$ instead of the calcium salt. The initial weight of the animals varied between 43-60 gm. At the end of 21 days both groups of animals showed severe rickets. The control group gained 25-45 gm. each while the strontium group gained 0-5 gm. An analysis of the serum of the control rachitic animals showed a Ca X P product of 15-24, while the strontium fed animals gave a product of 30 made up of Ca = 10.6 mg. % and Inorganic P = 2.8 mg. %.* The average ash content of the fat-free femurs was 33.23%. The flame test was negative for strontium in these bones.

To calcify the endochondral cartilage of the tibiae of each group, artificial serum solutions having a Ca X P product which varied from 30-60 were incubated. The technique of Shipley, Kramer and Howland[1] was used. The control group showed satisfactory calcification at products of 40, 50 and 60 while the tibiae from the strontium animals showed no fresh deposition.

The remaining animals of both groups were given 300 Steenbock units of vitamin D in the form of viosterol during a period of 9 days. All of the control rachitic animals healed and the serum Ca X P product rose to between 60 and 80. The untreated group still showed a Ca X P product between 15 and 25. The strontium animals failed to respond, the rickets being even more severe at the end of the 9 days of treatment than before therapy was begun. Nevertheless, the Ca X P product of the serum rose to 62. This value was due to a Ca of 12.1 mg. % and a serum inorganic P value of 5.1 mg. %.* The average ash content of the fat-free femurs was 29.4%, therefore decalcification of the bones progressed. The flame test was negative for strontium.

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* The possibility that both calcium and strontium were precipitated by oxalate has been considered. The quantitative separation of these elements is now being undertaken.

[1] Shipley, P. G., Kramer, B., and Howland, J., *Biochem. J.*, 1926, **20**, 379.

The results of *in vitro* calcification studies were the same as of above, the control group calcified at Ca X P of 60 while the strontium treated rats failed to heal.

Ten animals were used for strontium rickets and 10 for the control rickets. Six animals of each group were examined at the end of 21 days, 4 animals of each group at the end of 30 days. The strontium group received 300 Steenbock units of vitamin D while the controls examined did not.

Conclusions. The substitution of strontium for calcium in the rickets-producing diet produces a form of rickets which fails to respond to the very large amounts of vitamin D. The endochondral cartilage of strontium-fed animals both treated and untreated fails to calcify when incubated in artificial serum solution even with a Ca X P product of 60 which produces marked calcification in the endochondral cartilage of control rachitic rats. These observations strongly suggest an injurious effect of strontium upon the "local factor" in the hypertrophic cartilage of the provisional zone of calcification which brings about calcification at this area when conditions in the serum are favorable. The ability of vitamin D to cause an increase of the Ca X P even when healing does not take place, implies that this raising of the product is the function of the antirachitic vitamin in the cure of rickets.

7354 C

Effect of Ascorbic Acid on Thyroid and Suprarenals of Guinea Pigs.*

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From the Laboratory Division, Montefiore Hospital, New York City.

In previous publications from this laboratory[1-5] it has been shown that fresh plant and fruit juices contain an antigoitrous agent when

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* Aided by a grant from the Ella Sachs Plotz Foundation.

[1] Marine, D., Baumann, E. J., Webster, B., and Cipra, A., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 1025.

[2] Marine, D., Baumann, E. J., and Webster, B., PROC. SOC. EXP. BIOL. AND MED., 1930, **27**, 1029.

[3] Marine, D., *Ann. Int. Med.*, 1930, **4**, 423.

[4] Webster, B., Marine, D., and Cipra, A., *J. Exp. Med.*, 1931, **53**, 81.

[5] Marine, D., Baumann, E. J., Webster, B., and Cipra, A., *J. Exp. Med.*, 1933, **57**, 121.

tested on immature rabbits maintained on a goitrogenic diet of alfalfa hay and oats. (The goitrogenic effect of this diet is greatly enhanced by cyanide.) It was shown that this antigoitrous effect was in general inversely proportional to the iodine reducing capacity and that treating the fresh juice with copper salts destroyed or lessened this antigoitrous effect. Our data further indicated that the antigoitrous agent was probably identical with ascorbic acid but because of the lack of crystalline ascorbic acid on the one hand and the presence of iodine (also an antigoitrous agent), on the other, in all these extracts we were unable to prove it.

About 2 years ago one of us[6] simplified Szent-Györgyi's and Waugh and King's method of extracting ascorbic acid and by utilizing iris leaves, which are extremely rich in ascorbic acid, has prepared sufficient quantities of this hormone in crystalline form for these experiments, which were carried out as follows: Young guinea pigs weighing between 150 and 250 gm. were placed on a diet of alfalfa hay and rolled oats. (This diet produces scurvy in from 15 to 20 days. Pigs maintained on this diet when injected with the thyrotropic factor develop more marked exophthalmos and thyroid hyperplasia and more quickly than when given greens daily in addition.) They were injected with ½ to 2 cc. of ox anterior pituitary extract prepared after Loeb and Bassett's method and given 25 to 100 mg. of crystalline ascorbic acid by mouth daily. The pigs were sacrificed at various intervals and the principal data of 22 are given in Table I.

The thyroids of those given ascorbic acid and the thyrotropic factor average about 30% smaller than their litter mates given the thyrotropic factor alone. It also prevented or greatly lessened the hypertrophy of the suprarenal cortex which normally occurs in scurvy or following the administration of this preparation of the thyrotropic factor. Histologically there was less and in some cases no hyperplasia of the thyroid in those treated with ascorbic acid, although the presence or absence of hyperplasia depends on the proportion of ascorbic acid and the thyrotropic factor given as well as on the duration of the experiment. The guinea pig thyroid reacts quickly to the thyrotropic factor, while ascorbic acid acts more slowly so that in 4 days there may be a marked hypertrophy, while by the 8th day it may have disappeared completely if the proper doses of ascorbic acid and the thyrotropic factor have been given. Our data indicate that ascorbic acid directly or indirectly counteracts

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[6] Baumann, E. J., and Metzger, N., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1268.

TABLE I.

G. P. No. Series Sex		Wt. at beginning	Wt. at end	Duration of Exper.	Thyrotropic Factor Daily dose	Thyrotropic Factor No. days	Ascorbic Acid Daily dose	Ascorbic Acid No. days	Wt. of Thyroid	Wt. of Adrenals	Wt. of Pituitary	Scurvy	Thyroid Histology
		gm.	gm.	days	cc.		mg.		mg.	mg.	mg.		
Series 239													
23	F	215	253	20	2	12	25	8	65	155	9		Colloid
24	F	210	275	20	2	12			127	187	9	?	Sl. hypertrophy
25	M	187	229	20	2	12	25	8	90	130			Colloid
26	F	220	234	20	2	12	25	8	49	159	7		,,
27	M	234	264	20	2	12			71	114	9	+	,,
28	F	217	220	20	2	12			104	165	9	+	Sl. hypertrophy
Series 274													
121	F	169	169	6	1	6	100	6	55	99	5		Early ,,
122	F	211	212	7	1	7	100	7	70	141	5		Mod. mkd. ,,
123	M	197	232	13	1 0.5	7 5	100	12	40	105	8		Colloid
124	F	211	242	13	1 0.5	7 5			64	167	8		Mod. hypertrophy
125	F	209	213	7	1	7			59	149	5		Mkd. ,,
126	M	166	165	6	1	6			95	109	6		Mod. mkd. ,,
Series 275													
127	F	199	216	8	0.5	8	100	8	32	118	8		Colloid
128	M	244	303	14	0.5	13	100	13	42	133	8		,,
129	M	166	181	20	0.5	19	100	19	26	163	8		,,
130	M	173	205	20	0.5	19			34	148	7	+	,,
131	M	237	263	14	0.5	13			59	148	8	?	Mod. mkd. ,,
132	F	188	196	8	0.5	8			46	125	5		Mod. mkd. ,,
Series 278													
137	M	193	216	4	0.5	4			47	81	5		Early mod. ,,
138	F	194	201	8	0.5	8			44	125	8		Mod. ,,
141	F	168	200	8	0.5	8	50	8	39	108	7		Early mod. ,,
142	M	205	229	4	0.5	4	50	4	47	75	7		,, ,, ,,

the thyrotropic factor. This protective action of ascorbic acid against thyroid hypertrophy can be correlated with earlier work[7, 8] on (a) the production of a transient Graves' disease-like syndrome in rabbits and cats by sufficient but sublethal injury of the suprarenals; (b) with the sharp increase in the heat production of infants beginning on the 8th day after birth and associated with involution of the suprarenal cortex[9]; (c) with the beneficial effects of suprarenal cortex emulsion in the treatment of Graves' disease[10]; (d) with the metabolism lowering effect of fresh glycerol emulsions of suprarenal cortex emulsions in rabbits; and (e) with the association of Graves' disease with prolonged deep X-ray therapy to the suprarenal region in man.[11]

7355 P

Susceptibility of Heart to Electric Shock in Different Phases of the Cardiac Cycle.

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In connection with an extended series of experiments on the effects of electric shock, the fact has been brought to light that shocks whose duration is a small fraction of the cardiac cycle have a marked difference in their tendency to cause ventricular fibrillation, depending upon the phase of the cycle in which they occur. The shocks were tripped off by amplified cardiac currents after a controlled delay from a reference point in the electrocardiac cycle. The electrocardiogram together with the shock current and voltage were photographically recorded in time relation on a continuously moving film, which enabled checks to be made on the placement of the shocks in the cardiac cycle. Nearly 800 shocks with 60-cycle current of .03 and .1 second durations were administered to 92 anesthetized sheep in different parts of the cardiac cycle, with currents ranging from 1 to 17 amperes, through the pathway between

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[7] Marine, D., and Baumann, E. J., *Am. J. Physiol.*, 1921, **57**, 135.

[8] Scott, W. J. M., *J. Exp. Med.*, 1922, **36**, 199.

[9] Marine, D., Lowe, B., and Cipra, A., *J. Metabol. Res.*, 1922, **2**, 329.

[10] Shapiro, S., and Marine, D., *Endocrinology*, 1921, **5**, 699.

[11] Marine, D., *Am. J. Med. Sci.*, 1930, **180**, 767.

the right fore-leg and the left hind-leg. A large proportion of the shocks which were given near the end of ventricular systole, as determined by their coincidence with the T-wave of the electrocardiogram, caused ventricular fibrillation and death, while but one shock (0.1 second duration) distinctly outside the T-wave resulted in fibrillation.

It seems probable that the difference in the susceptibility of the heart to fibrillation from shocks in different phases of its cycle is related to the known variation of its excitability. Further investigation is being carried forward.

7356 P

Estimation of Small Traces of Alkaloids.

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From the Evans Memorial of the Massachusetts Memorial Hospitals, and Boston University School of Medicine.

The need to measure the amount of cocaine penetrating to the aqueous humor of an experimental animal after instillation of a cocaine solution into the conjunctival sac led to the following analytical procedure, which we find applicable to the quantitation of traces of many alkaloids in aqueous solution, and of certain alkaloids, particularly cocaine and strychnine, in body fluids. The method, which is accurate within the limits of colorimetric observations, is strikingly simple, and we believe will be of interest to workers in pharmacology, toxicology and experimental medicine.

A. *Procedure for aqueous solutions.* To a measured amount of solution containing from .5 to .05 mg. of the alkaloid is added 1 cc. of a 1% solution of phosphomolybdic acid in normal sulphuric acid. This is best done in a small test tube of about 5 cc. capacity. After mixing and standing for 15 minutes, the mixture in the test tube is centrifuged and the supernatant fluid decanted. The residue is resuspended in water, again centrifuged and the supernatant discarded. To the washed precipitate is now added 1 cc. of fifth normal sulphuric acid, 1 cc. of 2% aqueous solution of hydroquinone, and after shaking, 1 cc. of the carbonate-sulphite solution used in the Bell and Doisy[1] method for phosphate determination (15% sodium carbonate and 3% sodium sulphite). The color developed is read promptly in the small Duboscq type colorimeter against a standard prepared by treating 1 cc. of a solution of known and approximately equal alkaloid content (using the same alkaloid that is being determined) simultaneously with the same reagents. If necessary, it is a relatively simple matter to prepare several standards (5 of the small test tubes can be spun in an ordinary 50 cc. metal centrifuge cup) and the proper one can be selected by inspection.

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[1] Bell and Doisy, *J. Biol. Chem.*, 1920, **44**, 55.

B. *For body fluids of low protein content.* To a measured amount, 1 cc. or less, of aqueous humor or cerebrospinal fluid suspected of containing a certain alkaloid, is added 1 cc. of 20% sulphosalicylic acid. The protein is separated by the centrifuge, and the clear supernatant fluid removed by decantation and treated as in the procedure for aqueous solutions.

C. *For fluids of high protein content.* It is advisable to dilute blood serum with 4 volumes of physiological salt solution before deproteinization, in order to minimize the error resulting from adsorption of the alkaloid on the precipitated protein.

Results. With aqueous solutions of brucine, strychnine, cocaine, atropine, homatropine, sparteine, procaine, quinidine, caffeine, physostigmine, morphine, codeine, pilocarpine and apomorphine the analytical error was less than 6% if more than .1 mg. was present. With amounts from .05 to .1 mg., the error increased with decreasing amounts up to 20% at .05 mg., which is about the limit of possible colorimetric reading.

With solutions of cocaine or strychnine salts in aqueous humor or cerebrospinal fluid the analytical error is about the same in the weaker solutions, and up to 9% in the stronger. With blood serum, the error is up to 12%. With morphine there appears to be a marked tendency for the alkaloid to adsorb on the precipitated protein (it is not precipitated from simple aqueous solution by sulphosalicylic acid), hence this method is not applicable to morphine in body fluids.

7357 P

Effect of Parathormone on the Neuromuscular System.*

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From the Department of Physiology, University of Illinois, College of Medicine.

The present paper deals with an analysis of the action of parathormone on the neuromuscular system which may be considered the main seat of the tetany syndrome (MacCallum and Vogel[1]) The hind legs of pithed *Ranae esculentae* were perfused with phosphate buffered Ringer and the sciatic nerve stimulated with rhythmic condensor discharges as described previously.[2] After a state of relative fatigue had been reached in which the height of contraction remains equal over a long period of time, the perfusion fluid was exchanged for one containing parathormone, Lilly or Parke Davis, in concentrations from 1:1000 to 1:100. In all solutions the pH was carefully adjusted to that of Ringer. The phenol content proved to be without effect as control experiments indicated. In several hundred experiments parathormone invariably causes an increase in the height of contractions up to about 200% in the strongest concentrations. The effects are reversible when the solution is again exchanged for ordinary Ringer and the effects are due to the parathormone content of the extract for the following reasons:

1. Extracts of the parathyroid which according to the blood calcium test show the same number of units per cc. but contain solids of varying concentrations show an equal effect on the muscle even when the concentration of the solids varies 100%.
2. Boiling for one hour destroys neither the action of parathormone on the blood calcium (Collip and Thomson[3]) nor our reaction.
3. Destruction of the hormone content of the extract by boiling with 10% HCl for one hour abolished the described reaction on the muscle, as it does the blood calcium effect according to Collip.

The point of attack in our reaction is the neuromuscular junction. If, on account of the perfusion with curare 1:50,000 in Ringer, the neuromuscular junction is blocked, parathormone is without effect on the muscular contraction (direct stimulation). If, how-

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* Aided by a grant of the National Research Council and of the Graduate School of the University of Illinois.

[1] MacCallum, W. Gand, and Vogel, K. M., *J. Exp. Med.*, 1913, **18**, 618.

[2] Gellhorn, E., *Am. J. Physiol.*, 1933, **105**, 353.

[3] Thomson, D. L., and Collip, J. B., *Physiol. Rev.*, 1932, **12**, 309.

ever, the curare is washed out with Ringer's the effect reappears *pari passu* with the gradual reestablishment of the neuromuscular junction as indicated by the increasing height of the muscular contraction in indirect stimulation. The reaction is fine enough to serve in the assay of parathormone in conjunction with the ordinary blood calcium method. The parathormone effect is weakened by reducing the $CaCl_2$ content of the perfusion fluid. $CaCl_2$ can be replaced partially by $SrCl_2$. $CaCl_2$ also evokes an increase in the height of contraction in the perfusion preparation. Therefore, the question was examined as to whether parathormone influences the calcium effect. It was found that parathormone in a concentration which in itself did not influence the height of contraction increased the effect of calcium considerably. The height of contraction is considerably more increased by $CaCl_2$ in the presence of ineffective parathormone concentrations than in its absence. Such an effect of parathormone seems to be absent in the heart.

7358 P

Effect of Ethanol upon Fermentative Activity of Zymin.

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In studies on the effect of the composition of the medium upon the cell-free alcohol fermentation by zymin, it was soon evident that the zymin is very sensitive to relatively low concentrations of

TABLE I.

Relative rates of CO_2 production by yeast (from data of Rahn) and by zymin in the presence of varying concentrations of ethanol. Figures in () read from graph.

% Ethanol	Yeast	Zymin	% Ethanol	Yeast
0	100	100	4.93	60
0.38	(97)	89	5.48	55
0.94	(93)	75	7.40	38
1.31	(90)	61	9.55	24
1.37	87	(62)	9.70	29
1.86	(85)	50	9.86	27
2.59	(80)	43	11.9	16
2.74	77	(38)	12.1	13
3.65	(71)	28	13.1	14
4.11	65	(28)	13.3	15
4.70	(63)	28	14.3	12
4.77	70	—		

ethanol. In Table I are given quantitative data on the effect of various concentrations of ethanol upon the relative rates of fermentation based upon the production of carbon dioxide. There are also given the data obtained by Rahn[1] for yeast. An analysis of the latter data shows a linear relation between the concentration of alcohol and the relative rate of CO_2 production, up to a concentration of about 10%. From this point there is a slow and practically constant rate even in the presence of as high as 14%. There is a linear relationship for the zymin up to about 1.8% alcohol from which point there is also evident the low but continuous fermentation. The equations for the linear portions of the curve are

$$\text{Relative rate for yeast} = 100 - 2.1 \times \%\ \text{ethanol.}$$
$$\text{Relative rate for zymin} = 100 - 22.5 \times \%\ \text{ethanol.}$$

It is at once evident that the zymin is much more sensitive to the alcohol than is the yeast. Harden[2] reports that there is a 0-20% diminution of the fermentative activity of yeast juice in the presence of a 6% ethanol and a 75% decrease for 14% ethanol. In the preparation of zymin there is evidently removed some factor which protects against alcohol. The addition of the extracted material did not decrease the sensitivity. Experiments, the details of which will be published later, show that while the addition of phosphate gives the expected increase in activity there is no decrease in sensitivity toward ethanol; the addition of the appropriate concentration of ammonium chloride not only increases the fermentative activity, with or without the addition of phosphate, but very materially decreases the sensitivity toward ethanol.

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[1] Rahn, Otto, *J. Bact.*, 1929, **18**, 207.

[2] Harden, A., Alcoholic fermentation.

7359 C

Leucocytes in the Saliva in Normal and Abnormal Subjects.

D. J. STEPHENS AND EDGAR JONES.* (Introduced by W. S. McCann.)

From the Department of Medicine, University of Rochester School of Medicine, and the Medical Clinic of the Strong Memorial and Rochester Municipal Hospitals, Rochester, N. Y.

During a study of the physiologic variations of the white blood cells of normal individuals[1] in which enumerations of the circulating leucocytes were made at 15 minute intervals over a period of several hours, simultaneously the salivary leucocytes were counted in an attempt to correlate and interpret changes which might be observed. In all, 367 such observations were made of 20 normal individuals. The observations were then extended to 145 determinations of 17 patients with a variety of pathological conditions. The salivary leucocytes were counted according to the method described by Isaacs and Danielian.[2] In making white blood cell counts of blood drawn from the ear, the method previously described[1] was used, in which, by counting 4 times the usual number of cells, the probable error was found to be about ±230 cells.

Our observations of the number of salivary leucocytes in normal individuals are in essential agreement with those of Isaacs and Danielian. In more than 50% of the subjects, the number of salivary leucocytes was below 25 cells per cu. mm.; counts of from 500 to 1000 or more cells per cu. mm. were occasionally observed.

However, in our data, there is no apparent correlation between the number of the white cells in the blood and those in the saliva. The salivary leucocyte counts were fairly uniformly scattered over a series of individuals whose range of white blood cell counts was from 4,000 to 12,000 per cu. mm. Marked fluctuations were found in the salivary count without any significant change in the leucocytes of the peripheral blood and vice versa. When fluctuations occurred in both counts during the same interval there was no consistent relationship of one to the other. Neither in individuals nor in the entire group was there evidence of a consistent reciprocal relationship between the numbers of leucocytes in the saliva and peripheral blood.

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* This investigation was aided in part by a grant from the Josiah Macy, Jr., Foundation.

[1] Jones, E., Stephens, D. J., Todd, H., and Lawrence, J. S., *Am. J. Physiol.*, 1933, **105**, 547.

[2] Isaacs, R., and Danielian, A. C., *Am. J. Med. Sci.*, 1927, **174**, 70.

Salivary counts and white blood cell counts were taken simultaneously in 5 cases of myelogenous leucemia, during treatment with Roentgen ray irradiation and with Fowler's solution. Marked fluctuations which occurred in the white blood cell counts of these individuals were not accompanied by any consistent change in the number of salivary leucocytes. Further, no constant relationship between leucocytes of saliva and peripheral blood were seen in observations made in other pathological conditions, including agranulocytic angina, thrombocytopenic purpura with chronic granulocytopenia, carcinoma of stomach, carcinoma of breast, Hodgkin's disease, lymphatic leucemia and lobar pneumonia.

Similar conclusions were reached by Elliott and Jenkinson,[3] who followed the saliva of patients with myelogenous leucemia treated with irradiation.

Conclusions. In a series of 512 enumerations of leucocytes in saliva and peripheral blood in 20 normal and 17 abnormal individuals, no correlation has been observed between the total number of white blood cells in the peripheral blood and in the saliva.

7360 C

Comparative Pharmacology of Isomeric Saligenins and Their Bromine Derivatives.

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From the Chemical and Pharmacological Research Laboratories, Hynson, Westcott & Dunning, Inc., Baltimore.

Macht and Dunning described the chemical and physiological properties of mono-brom-hydroxy-benzyl alcohol, or mono-brom-saligenin.[1] This compound, which has been named *Bromsalizol,* was the mono-brom derivative of ortho-hydroxy-benzyl alcohol. Inasmuch as 3 isomers of saligenin with the hydroxyl OH and benzyl CH_2OH groups in the ortho, meta and para positions, respectively, are possible, they and their mono-brom derivatives were prepared in these laboratories and investigated with regard to their comparative pharmacology. The structural formulae of the 3 saligenin isomers and their corresponding mono-brom derivatives are as follows:

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[3] Elliott, A. B., and Jenkinson, E. L., *Med. Clinics of N. Am.*, 1933, **17**, 327.

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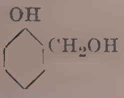
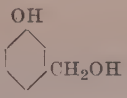
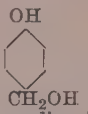


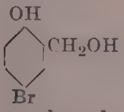
ortho-saligenin



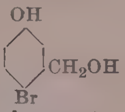
meta-saligenin



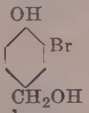
para-saligenin



2 oxy-5 brom-benzyl alcohol
(Bromsalizol)



mono-brom-meta-
saligenin



mono-brom-para-
saligenin

The melting point of ortho-saligenin was 85°-87°; of meta-saligenin, 70°-71°; of para-saligenin, 116°; of mono-brom-ortho-saligenin, 108°-109°; of mono-brom-meta-saligenin, 124°; and of mono-brom-para-saligenin, 128°.*

A pharmacodynamic comparison of the compounds mentioned above was made by studying (1) their effect and that of controls on the growth of *Lupinus* seedlings in plant physiological solutions; (2) their action on the muscular coordination and respiration of goldfish, *Carassius auratus;* (3) their influence on the behavior of mice, injected intraperitoneally; and (4) of rats, also injected intraperitoneally; (5) their local anesthetic effect on the cornea of the rabbit's eye and on frog-skin; (6) their action on surviving preparations of smooth muscle from the intestine of the cat and uterus of the guinea pig; (7) on the kidney function of rabbits after administration by mouth; (8) on the circulation and respiration of etherized cats after intravenous injection; (9) their antipyretic effect on rabbits and rats; and ascertaining (10) the killing intravenous dose for cats. Most of the results obtained are summarized in Table I.

It was found in each case that the mono-brom-saligenins were more potent pharmacologically than their corresponding mother saligenins. The di-brom-saligenins and other halogen derivatives are under investigation and will be described later. Most of the physiological reactions obtained demonstrated that the ortho-saligenin was more active than the meta-saligenin and the meta- more active than the para-saligenin. Similarly, the brominated ortho-saligenin was more active than the brominated meta-saligenin, and the latter was more potent than the brominated para-saligenin. The single exception to this rule was the effect produced on smooth

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* Complete data regarding the chemical and physical properties of these compounds are to be published by one of the authors (H. A. B. D.) in his dissertation for a doctorate in chemistry.

TABLE I.

Pharmacology of Saligenin and Brom-Saligenin Isomers.**

Compound	Effect on *Lupinus Albus* Seedlings of Solutions of		Effect on Goldfish, *Carassius Auratus*		Effect on Mice of Intraperitoneal Injections of	Dose Inhibiting Contractions of Intestinal Segment mg.	Anesthesia of Frogskin†	Minimal Lethal Dose on Intravenous Injection in Cats mg. per kilo
	1: 5,000 %	1: 10,000 %	Inco-ordination	Respiratory paralysis				
Ortho-saligenin	27 (20)	52 (20)	15 min. 1:10,000 (10)	50 min. 1:10,000 (10)	15 mg. coma in 5 min.; dead in 2 hours (10)	50 (15)	.200 (20)	532 (2)
Meta-saligenin	62 (10)	77 (10)	no effect 1:2,500 (6)	no effect 1:2,500 (6)	30 mg. coma in 20 min. recovery in 2 hours (6)	45 (6)	.040 (5)	615 (2)
Para-saligenin	81 (10)	90 (10)	no effect 1:2,500 (6)	no effect 1:2,500 (6)	30 mg. coma in 25 min. recovery in 1 hour (6)	65 (6)	.006 (5)	934 (2)
Brom-ortho-saligenin, Bromsalizol	22 (20)	50 (20)	25 min. 1:10,000 (10)	80 min. 1:10,000 (10)	10 mg. coma in 2 min. dead in 20 hours (6)	3 (15)	1.000 (20)	250 (2)
Brom-meta-saligenin	41 (10)	80 (10)	62 min. 1:10,000 (6)	120 min. 1:10,000 (6)	10 mg. semiconsciousness in 14 min. recovery in 1½ hrs (6)	2 (6)	.400 (5)	153 (2)
Brom-para-saligenin	71 (10)	100 (10)	120 min. 1:10,000 (6)	150 min. 1:10,000 (6)	20 mg. slight depression in 20 min. recovery in 1 hour (6)	30 (6)	.170 (5)	361 (6)

** Figures in parenthesis indicate number of experiments.

† The anesthetic efficiency of Bromsalizol being regarded as 1.000, the relative efficiency of the other compounds is expressed as decimals of that standard.

muscle. The brominated meta-saligenin was more powerful than the brominated ortho-saligenin. On the other hand, the toxicity of the brom-meta-saligenin for cats was considerably greater. None of the saligenins or of their brominated derivatives produced impairment of the kidney function of rabbits after administration by stomach tube of as much as 250 mg. per kilo weight. The saligenins themselves exhibited a slightly antipyretic effect on rabbits in which hyperpyrexia had been produced by injections of hay infusion. The brominated compounds, however, showed a definite antipyretic effect in such animals. Experiments with injections of camphorated oil revealed that the brominated saligenins, and especially Bromsalizol, antagonized the convulsant

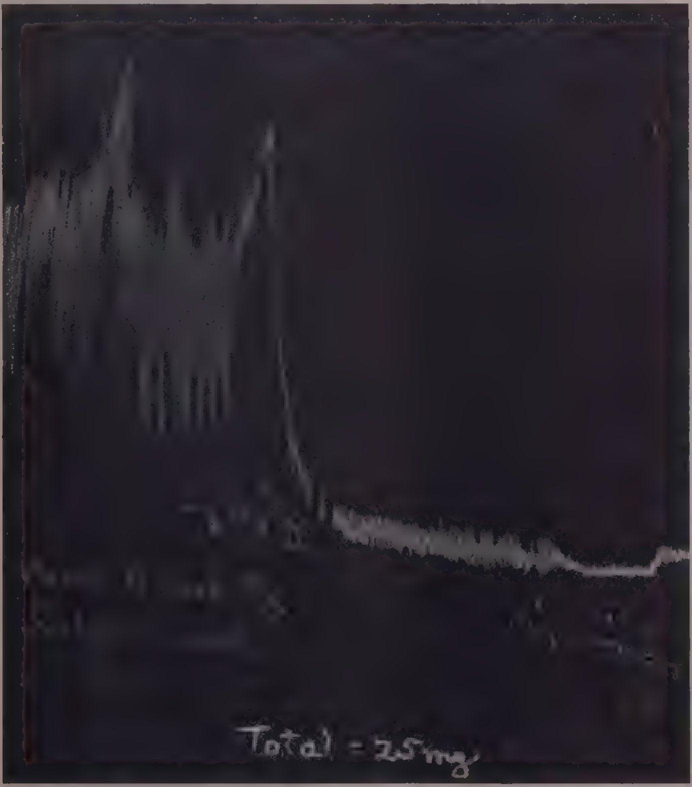

FIG. 1.

Preparation of uterine muscle from the guinea pig. Effect of repeated doses of ortho saligenin in 50 cc. of Locke's solution until complete inhibition of contractions results.

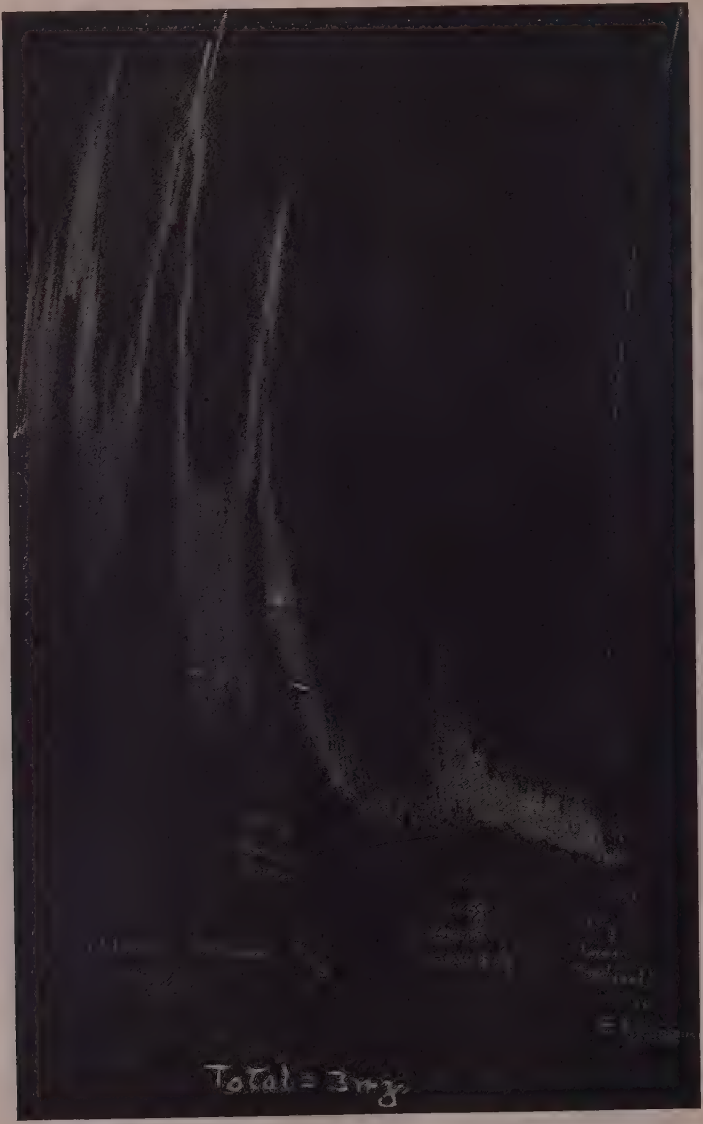

FIG. 2.
Preparation of uterine muscle from guinea pig. Effect of mono-brom-ortho saligenin, or Bromsalizol. 3 mg. in 50 cc. of Locke's solution produce inhibition of contractions but not death, as is indicated by response of preparation to pilocarpine.

effects of camphor injections in rats and guinea pigs. Such an antagonism was not produced by ortho-saligenin itself, thus indicating that the brominated compound produced some sedation of the cerebrum.

The comparative study of the two kinds of isomers mentioned above strikingly emphasizes the selective, quantitative differences in the action of chemical isomers. Figures 1 and 2 illustrate the effect of ortho-saligenin and its mono-brom derivative, Bromsalizol, on surviving preparations of the guinea pig uterus. It will be observed that the brominated compound, as an antispasmodic, is 8 times more

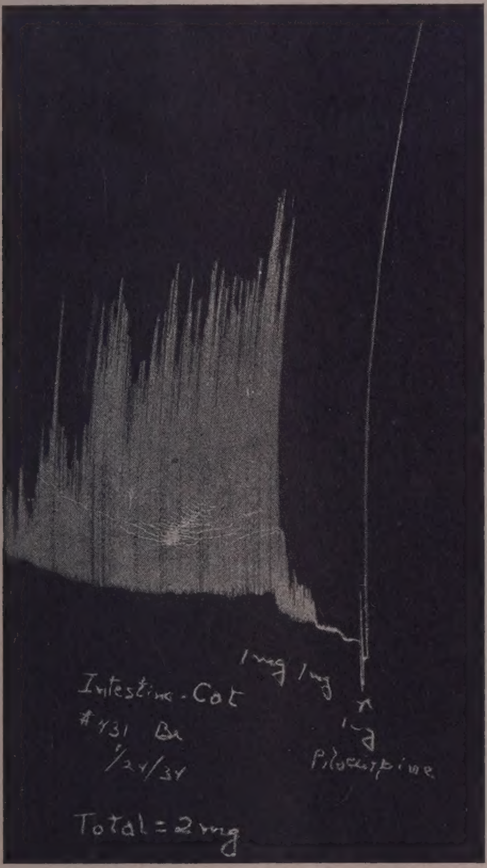

FIG. 3.
Intestinal segment of cat. 2 mg. of mono-brom-meta saligenin in 50 cc. of Locke's solution inhibit contractions.

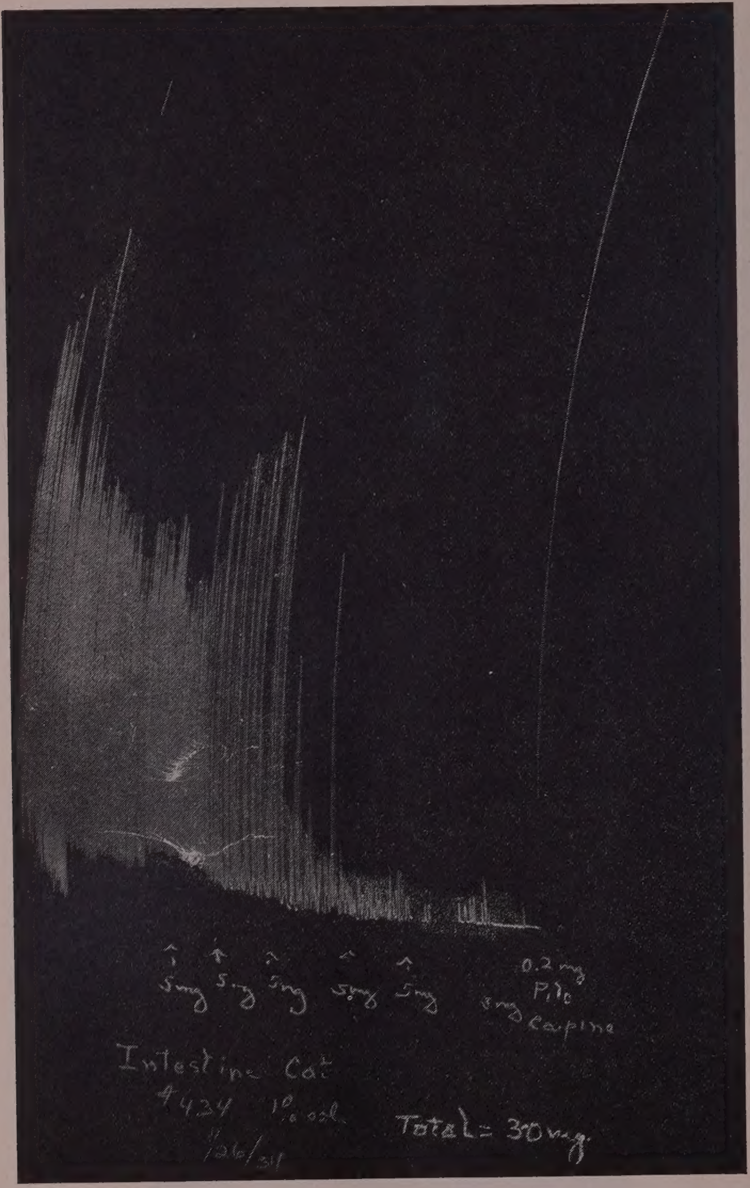

FIG. 4.

Intestinal preparation of cat. Effect of mono-brom-para saligenin. It required 30 mg. in 50 cc. of Locke's solution to inhibit contractions.

powerful than saligenin itself. Figures 3 and 4 illustrate the difference between the effects of brom-meta-saligenin and brom-para-saligenin on isolated intestinal segments from the same cat. Again, it will be noted that the brom-meta compound is 15 times more potent than the corresponding brom-para compound as a sedative for smooth muscle. Clinical studies have already been made with regard to the antispasmodic effects of the above mentioned compounds and are now being conducted especially with mono-brom-ortho-saligenin, or Bromsalizol, which, as appears from the table, offers the widest margin of safety as well as the most efficient therapeutic index.

7361 C

Vitamin A of Serum Following Administration of Haliver Oil in Normal Children and in Chronic Steatorrhea.

JACK CHESNEY AND AUGUSTA B. McCOORD. (Introduced by S. W. Clausen.)

From the School of Medicine, University of Rochester, New York.

After the ingestion of an oil of high vitamin A content, such as haliver oil, a marked increase in the intensity of the Price-Carr reaction for vitamin A is observed in the blood serum of normal persons. The degree of this increase may indicate the ability of the individual to absorb vitamin A. The amount of vitamin A in the serum is stated in colorimetric units which are purely arbitrary. Using a 10% solution of copper sulphate as a standard, serum of sufficient content in vitamin A to match the standard when acted upon by the antimony trichloride reagent is considered to contain 100 units of vitamin A per 100 cc. Each colorimetric unit is equivalent to 2.9 biological units.

To 12 afebrile children who were considered to have no disorder in the absorption of fats, 2 cc. of haliver oil* were administered while the children were fasting. Throughout the remainder of the 24-hour test period, the children ate meals with low vitamin A content at the usual times. Blood was taken for analysis before the ingestion of the oil, and 2, 4, 6, 9, 12, and 24 hours afterwards. The maximum concentration of vitamin A is observed at 4 hours and is approximately 9 times the fasting level. (Table I.) The level at 24 hours is about 50% above the original level.

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* Furnished by the courtesy of the Abbott Laboratories.

Two children suffering from idiopathic steatorrhea (celiac disease) were observed during the course of their hospitalization and treatment. It was found that the rise of vitamin A in the serum is much less than in the normal children and that the maximum rise occurs later after the ingestion of the oil; also, that during the phase of clinical improvement, the rise is more marked. We may assume that the low rise is due to faulty absorption although a more rapid withdrawal from the blood stream or a reexcretion into the intestine may play a part.

TABLE I.

Time	Normal		Celiac Disease				Chronic Diarrhea (Not Celiac)	
			Case I		Case II			
hr.	Mean	Probable error	Before Treat.	After Treat.	Before Treat.	After Treat.	Case I	Case II
Fasting	25.4	± 2.05	12	14	8	28	28	48
2	72.8	± 11.0	20	14	11	34		73
4	226.8	± 13.8	37	100	8	21	300	294
6	153.5	± 15.1	45	146	12			275
9	71.0	± 2.9	80	52	11	45		75
12	55.1	± 2.2	74	30	15		49	69
24	37.5	± 3.6	39	22	14	37		31

In 2 patients with chronic diarrhea, stools with abnormally high fat content and a flat glucose-tolerance curve, all of which are suggestive of celiac disease, the vitamin A absorption curve was found to be normal. The subsequent clinical course of these patients proved that they were not suffering from celiac disease. It is, therefore, suggested that the test for vitamin A absorption may have diagnostic value in the chronic diarrheas.